An Evaluation of the Technicon RA-1000 Random-Access Analyzer
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We evaluated the Technicon RA-1000 analyzer, with emphasis on its potential for user-defined method development. Optical linearity and sample pipetting linearity were good. The reagent pipetting system delivered slightly less than the nominal amount, owing to the volumetric effect of added mixing bubbles. Carryover of aqueous solutions was negligible. The instrument had good adaptability for user-defined methods and performed well in method comparisons. The observed dynamic range for enzymes (0–3000 U/L) was excellent. The working software performed its intended functions well, but has limitations. We believe that the RA-1000 represents a significant contribution to the practice of clinical chemistry. A sophisticated benchtop machine, it includes several innovations, along with a few problems that are peculiar to its technology.

Additional Keyphrases: method development and evaluation - kinetics - carryover - polytetrafluoroethylene - molar absorptivity

The Technicon RA-1000 "random-access" discrete analyzer became commercially available in the summer of 1982. Technicon Instruments Corporation (Tarrytown, NY 10591) invited us to evaluate one of the first production units. Our objectives, in addition to the usual testing of analytical instrument performance, were to see how well methods in routine use in our laboratory could be adapted to this machine.

After an initial six-month evaluation that began in mid-September 1982, with all related costs met by Technicon, the RA-1000 was put into routine service, including online connection to our laboratory computer system.

General Observations

The RA-1000 has some novel features. The principles and performance of the random access pipetting system for samples and reagents have been described (1). A polyfluorinated hydrocarbon liquid called "Random Access Fluid" (RAFT) is used to coat the internal and external surfaces of polytetrafluoroethylene (Teflon) probes, to eliminate carryover.2 Controlled by the high-speed version of the Intel MCS-85 microprocessor and related peripheral controllers, with 64 kilobytes of memory (2), a sophisticated algorithm is used for reaction cuvette positioning, moving, mixing, and reading (3). The operating program, on a mini-floppy diskette, is easily updated and corrected; new versions of the program are distributed by the supplier.

The RA-1000 is "benchtop" size, with a heavy cast chassis. It weighs a total of 124 kg. It is mechanically quite simple and truly requires little routine maintenance. It is easily installed; only a standard power connection and no plumbing or waste drainage is required. Routine start-up is quick and simple.

Learning the routine operation of the machine is easy, but the keyboard is small and requires the entry of numeric "command function codes." The program provides for worklisting and programming of test profiles and of reagent-tray configurations. The ability to change the reagent tray configuration to accommodate the different workloads of day/evening/night shifts is convenient.

The instrument always performs reagent blanks and can optionally perform a sample blank. It can do endpoint (equilibrium), first-order kinetic (initial reaction rate), or zero-order kinetic (enzyme) methods. Calibration for substrate measurements is by single-level standards, run in replicate.

Materials and Methods

Standard reference potassium dichromate was obtained from the National Bureau of Standards, Office of Standard Reference Materials, Washington, DC. Solutions of it were prepared in 10 mmol/L HCl.

Technicon RA-1000 Random-Access Fluid, Wetting Agent W, and disposable reaction trays, reagent boats, sample cups, tubing, and probes were used. We used the 30 °C temperature setting.

We used Technicon RA-1000 reagent kits for urea and the tests listed in footnote 2.

In comparing performance of urea and CRE the Beckman Astra 8 was used with Beckman reagents (Beckman Instruments Inc., Brea, CA). The Technicon RA-1000 CRE reagent can only be used for 4 h after preparation, and so we freshly prepared it as required. The Beckman CRE is stable for one week and can be used on the RA-1000.

Other instruments compared with the RA-1000 included the Technicon SMA 12/60 for TPR and ALB, the Enzyme Analytical Centrifugal analyzer (Enzyme-Nucleonics, Inc., Fairfield, NJ) for CK, and the ABA-100 discrete batch analyzer (Abbott Laboratories, Diagnostics Division, Dallas, TX) for GLU, AST, ALT, and GT.

The bromcresol green and biuret reagents used in the SMA 12/60 for the ALB and TPR comparisons were prepared in-house according to Technicon formulations. Ortho Automated Reference Serum (Ortho Diagnostics, Don Mills, Ontario, Canada) was used to calibrate ALB and TPR in both the SMA 12/60 and the RA-1000.

The "A-Gent Glucose-UV" kit (Abbott Laboratories, Diagnostics Division, South Pasadena, CA 91030) was used in the GLU method comparison.

The "Auto/Stat" kit for inorganic phosphorus (Pierce Chemical Co., Box 117, Rockford, IL 61010) was compared with the Technicon RA-1000 P kit, both used in the RA-1000. The Pierce kit was run with 20-μL sample volume, without sample blanking, and the end point was monitored at 600 nm.

We used kits from Boehringer/Mannheim Canada Ltd., Dorval, Quebec; in method comparisons for GT (cat. no.

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The "StatZyme GOT" kit (Worthington Diagnostic Systems, Inc., Freehold, NJ 07728) was used in the AST method comparison.

Results

To evaluate the optical performance of the RA-1000 spectrophotometer, we used potassium dichromate standard solutions prepared with absorbances expected to range from 0 to 3.5 $\text{A}$ at 340 nm. These solutions were manually introduced into the cuvettes while the instrument ran. The within-run optical noise, expressed as the CV of the printed absorbance, was 0.2% or less over the range 0.7 to 3.4 $\text{A}$, increasing to about 0.6% at 0.15 $\text{A}$ (n = 8). Thus at 1.0 $\text{A}$ the noise was 0.002 $\text{A}$ or less. Figure 1 shows that the optical linearity extended all the way to 3.5 $\text{A}$, with good accuracy. The RA-1000 software has an absorbance cutoff which prevents it from reporting a result if the test absorbance is greater than about 1.8 $\text{A}$.

The three types of carryover possible in the RA-1000 were discussed in detail by Smith et al. (1). We could not detect carryover with any of the methods we evaluated and so tried water alternating with concentrated potassium dichromate solutions, measured at 340 nm. Still we could not detect reagent-to-reagent or sample-to-sample carryover, and although reagent-to-sample carryover was detectable it was definitely negligible.

Sample pipetting linearity was tested with ink solutions at sample volumes of 2, 4, 6, 9, 15, and 20 $\mu\text{L}$ in the calculations correction for the varying sample volume was included and the results were linear over this range ($r = 1.000$).

We tested reagent pipetting accuracy gravimetrically with correction for the weight of RAF delivered along with the reagent. By this technique the volume delivered by the reagent pipetting system was consistently found to be too low. At a nominal setting of 350 $\mu\text{L}$ the volume measured in two trials was 342 and 339 $\mu\text{L}$. Two months later, after new tubing was installed, these values were 331 and 332 $\mu\text{L}$. This effect results from the volume of the added reagent-mixing air bubbles, which are introduced into the reagent stream during the aspiration stroke of the reagent syringe.

We found the RA-1000 has a broad dynamic range for enzyme assays (0–3000 U/L), which by far exceeds Technicon’s claims for linearity, achieved by multiple readings taken at short intervals.

Fig. 1. Optical accuracy and linearity of the RA-1000 measuring potassium dichromate absorbance standards at 340 nm.

Because of space limitations we are omitting our RA-1000 analytical precision data. Others are reporting in detail on this aspect already (4, 5). However, we observed some problems peculiar to the RAF technology, which affect precision and deserve description:

Quite often the first result of a run is too low, caused by a short pipetted sample volume. When the RA-1000 is idle the sample probe rests in a reservoir of RAF, wetting its Teflon surface. At the start of a run the sample probe lifts up and momentarily an excess of RAF drips down its exterior. This can either form a large droplet at the tip of the sample probe or may drip off. If it remains at the tip it will then be sucked in before the next sample, causing short-sampling. On rare occasions the droplet falls to the bottom of the sample cup, and since the probe tip is within 2 mm of the bottom during aspiration, some of the RAF may be sucked in, again causing a short-sample. To avoid the "first test error" it is prudent to run one user-defined "dummy" test to prime each run.

Each time the sample probe dips into a specimen some RAF may be shed, sinking to the bottom. If several tests were requested for one specimen, enough RAF may accumulate at the bottom to cause short-sampling.

The RAF completely envelopes the sample during delivery into the reagent (1) and this envelope has to be disrupted to ensure complete mixing with reagent. Mixing can be incomplete if there is excess RAF (problem with the RAF peristaltic pump), if the sample probe tip is too far from the floor of the cuvette (which has angular ridges on it to help break the globule), or if the sample probe tip is torn or shredded (allowing the globule to squirt out laterally and remain intact), especially with small sample volumes (2–6 $\mu\text{L}$). When results are printed as zero or unusually low numbers, the operator could check the used cuvettes for RAF globules containing unmixed sample material.

Random high results occur if the air bubble solenoids for the reagent pipetting system operate improperly and allow varying excess air volume into the tubing (too little reagent delivered).

If care is taken to prevent these problems (e.g., by routinely inspecting the reaction tray after use), very good precision is obtained.

Table 1 summarizes our method comparison observations. AST and ALT, which are not shown in Table 1, gave "<0" results for about a third of sequential patients. These samples run on the ABA-100 gave results in the 5 to 20 U/L range. When the RA-1000 absorbance readings for AST and ALT were printed out for these low samples or when water was used as a sample, a slow upward drift was observed (these methods are supposed to be downgoing at 340 nm; i.e., the test absorbances should be decreasing during the analysis interval). This upward drift in RA-1000 absorbances occurred with other stable chromogens such as potassium dichromate solutions, and thus would appear to be caused by evaporation from the reaction tray during the analysis. Although this may seem surprising, it should be recalled that the reaction tray is constantly moving (causing a marked increase in effective surface area for evaporation because of reaction mixture wetting the walls of the cuvette above the surface of the fluid), is exposed to moving warm air during incubation, and has surfactants added to the reagent mixture. Method parameters can be programmed into the RA-1000 to partly compensate for this drift.

The small proportional error in the GT comparison is to be expected, because the indicator substrates are not the same.

For CRE, the expected and observed values were linearly related up to 1500 $\mu\text{mol/L}$ for a series of 20 aqueous standards prepared in 75 $\mu\text{mol/L}$ increments.
Table 1. Method Correlations

<table>
<thead>
<tr>
<th>Test</th>
<th>Range</th>
<th>Units</th>
<th>n</th>
<th>Correlation</th>
<th>r</th>
<th>S&lt;sub&gt;r&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU</td>
<td>2–25</td>
<td>mmol/L</td>
<td>128</td>
<td>ABA100 = 1.00 × RA1000 + 0.05</td>
<td>0.9980</td>
<td>0.3</td>
</tr>
<tr>
<td>Urea</td>
<td>2–32</td>
<td>mmol/L</td>
<td>26</td>
<td>RA1000 = 0.967 × Astra + 0.63</td>
<td>0.9977</td>
<td>0.5</td>
</tr>
<tr>
<td>CRE</td>
<td>30–1200</td>
<td>µmol/L</td>
<td>139</td>
<td>RA1000 = 1.02 × Astra − 4.5</td>
<td>0.9964</td>
<td>13</td>
</tr>
<tr>
<td>TPR</td>
<td>40–92</td>
<td>g/L</td>
<td>139</td>
<td>RA1000 = 1.00 × SMA − 0.3</td>
<td>0.9927</td>
<td>1.3</td>
</tr>
<tr>
<td>ALB</td>
<td>3–52</td>
<td>g/L</td>
<td>143</td>
<td>RA1000 = 1.01 × SMA − 0.3</td>
<td>0.9908</td>
<td>1.3</td>
</tr>
<tr>
<td>P</td>
<td>0.4–3.5</td>
<td>mmol/L</td>
<td>79</td>
<td>Technicon = 1.07 × Pierce − 0.01</td>
<td>0.9909</td>
<td>0.1</td>
</tr>
<tr>
<td>CK</td>
<td>10–650</td>
<td>U/L</td>
<td>57</td>
<td>GEMSAEC = 1.01 × RA1000 − 2.3</td>
<td>0.9985</td>
<td>6</td>
</tr>
<tr>
<td>GT</td>
<td>8–500</td>
<td>U/L</td>
<td>62</td>
<td>ABA100 = 1.23 × RA1000 − 4.8</td>
<td>0.9950</td>
<td>15</td>
</tr>
</tbody>
</table>

Discussion

We have experienced a few minor malfunctions of the RA-1000. The reagent probe tends to curl, eventually crashing into an edge and requiring replacement. Probe replacement is simple but adjusting for correct alignment takes a long time. Imprecision due to dripping fluid occurred if new probes were not carefully cleaned (as the manual instructs) in NaOH to remove manufacturing oil. The tip of the sample probe should be regularly inspected, under magnification, for micro-tears, since these can lead to poor sample-reagent mixing and hence deteriorating precision. The air-bubble solenoids failed and had to be replaced once (the symptom of failure was gross reagent pipetting imprecision).

The segmentation of the reagent stream introduced a small, relatively constant error in absolute volume delivery which can be compensated for in setting the calibration factor. If the system could be re-designed to introduce the bubbles after the full volume of reagent has been aspirated and the probe is clear of the reagent surface, this problem would be eliminated.

We endured much frustration from "bugs" in the software and much of our labor was spent defining the exact conditions under which malfunctions occurred. However, by the time we put the instrument into routine service in July 1983 the most substantial software bugs had been eliminated with a new version of the operating diskette, and Technicon had installed certain hardware upgrades to make our RA-1000 the same as the newer production units.

Although the worklisting features were effective and convenient to use for their intended purpose, there were limitations that tended to interfere with analytical throughput. Net throughput is lessened by separate sample blanks, the need to stop the machine to change the reaction tray when it is full, and the fact that certain keyboard interactions cannot be used while analysis is in progress, either because they are not allowed by the system or because it would interfere with the printing of results (thus the analytical part of the machine not infrequently is idle while keyboard commands are entered).

User-defined adaptability is very good and incorporates several innovative features that provide the flexibility and programmability desirable in an "ideal" all-purpose instrument. The operating manual gave inadequate documentation of user method development; however, later Technicon literature outlined the built-in method development aids and system details that make method adaptation straightforward.

The machine performed well in method comparisons, and for complete agreement with other systems, programmable slope, intercept, and drift parameters can be used.

Solutions in the reaction cuvettes dry up fairly rapidly due to the warm air incubator. This makes reaction tray clean-up and re-use seem impractical. In some methods, evaporation from the reaction cuvette may cause appreciable upward absorbance drift, particularly with long analysis time, high starting absorbance, small reagent volume, the use of added superfuctant, and operation at 37°C. The surface area available for evaporation is increased by the agitated stepping motion of the tray, which drives liquids up the side-walls of the cuvettes.

We conclude that the RA-1000 is a significant contribution to the practice of clinical chemistry. It introduces several innovative techniques and some unique problems. It is versatile, quite simple, adaptable, and reliable. Despite the fact that neither the sample nor the reagent probe is ever washed, carryover of aqueous solutions is virtually undetectable. Spectrophotometer performance was excellent. It performed well in equilibrium, initial rate, or enzymatic methods. Its multi-point approach to enzyme assays with a short analysis time achieves a very wide dynamic range on undiluted samples.

References

5. Sullivan A, Scanlon J, et al. (University of Vermont Medical Center Hospital, Burlington, VT), in preparation for publication.