Comparison of Two Dry Chemistry Analyzers and a Wet Chemistry Analyzer Using Canine Serum

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John W. Kramer

Canine serum was used to compare seven chemistry analytes on two tabletop clinical dry chemistry analyzers, Boehringer’s Reflotron and Kodak’s Ektachem. Results were compared to those obtained on a wet chemistry reference analyzer, Roche Diagnostic’s Cobas Mira. Analytes measured were urea nitrogen (BUN), creatinine, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, and bilirubin. Nine to 12 canine sera with values in the low, normal, and high range were evaluated. The correlations were acceptable for all comparisons with correlation coefficients greater than 0.98 for all analytes. Regression analysis resulted in significant differences for both tabletop analyzers when compared to the reference analyzer for cholesterol and bilirubin, and for glucose and AST on the Kodak Ektachem. Differences appeared to result from proportional systematic error occurring at high analyte concentrations.

Key Words: Serum chemistry, canine, dry chemistry analysis, serum chemistry analyzer

Introduction

With microcomputerization came the development of reflectance photometry and multilayer, thin film dry clinical chemistry analyzers. They are easily operated and offer a wide range of clinical screening tests that can be performed in an economical, timely, and convenient manner that meets the Clinical Laboratory Improvement Amendment’s testing complexity, certificate of waiver. Plasma or serum applied to dry surface is drawn by capillary action through an exclusion membrane into a dry gel or fiber pad containing immobilized reagents.1 Dry clinical chemistry analytical procedures are similar to the established wet clinical chemistry procedures. The gel or fiber pad becomes the reaction vessel similar to the cuvette of the wet chemistry analyzer. The color change in dry chemistry procedures is detected by reflected light photometry in contrast to transmitted light of wet chemistries.

The Reflotron (Boehringer-Mannheim Diagnostics, Indianapolis, IN) requires low maintenance, infrequent calibration, has a low cost per analyte, and is easy to use. It does not, however, enable analysis of some routinely performed veterinary chemistries such as SDH, calcium inorganic phosphorus, or sodium and chloride. It does have the advantage of assaying whole blood as well as serum or plasma for most analytes.

A whole blood sample is applied to a glass fiber pad. Capillary action draws the blood through the fiber filter, entraping the blood cells and allowing plasma to flow into a second pad containing immobilized dry reagents.1 Dry clinical chemistry analytical procedures are similar to the established wet clinical chemistry procedures. The gel or fiber pad becomes the reaction vessel similar to the cuvette of the wet chemistry analyzer. The color change in dry chemistry procedures is detected by reflected light photometry in contrast to transmitted light of wet chemistries.

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The Reflotron and Ektachem for use outside regular hospital hours and microlabs within our Veterinary Teaching Hospital. The clinical pathology laboratory’s wet chemistry analyzer, the Cobas Mira (Roche Diagnostic Systems, Inc. Nutley, New Jersey), constituted the reference analyzer for this

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Materials and Methods
Determinations were performed on canine serum for blood urea nitrogen (BUN), creatinine, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, and bilirubin. Samples were stored at -20°C. They were thawed and thoroughly mixed immediately prior to analysis. Nine to 12 samples were analyzed in duplicate for each biochemical analyte, including at least three samples with values in the low or high range, and three to five samples in the normal range. In order to obtain low canine serum values for glucose, AST, ALT, and cholesterol, an aqueous 6.5% bovine serum albumin solution was used to dilute normal canine serum. This solution was used rather than saline to ensure that a consistent protein matrix was maintained in the dry chemistry procedures. Commercial normal level control serum was assayed with the samples to determine within- and between-run precision of the assays. Determinations were performed over a period of 6 days. Statistical analysis included calculation of correlation coefficients, linear regression analysis, and the Student's t-test for evaluation of regression line slopes.

The serum chemistry procedures used by Reflotron (ALT, #745138; AST, #7451200; BUN, #1200704; cholesterol, #745065; creatinine, #88687; glucose, #7449484; total bilirubin, #9053210) and Ektachem (ALT, C-3636; AST, C-338; BUN, C-301; cholesterol, C-305; creatinine, C-353; glucose, C-300; total bilirubin, C-305) were: the diazotization salt procedure for total bilirubin, cholesterol esterase procedure for cholesterol, glucose oxidase procedure for glucose, and creatinine hydrolase for creatinine. The wet chemistry procedures used for the BUN, total bilirubin, and cholesterol were basically the same as those of the dry chemistry procedures, but the hexokinase procedure was used for glucose and the Jaffe reaction for creatinine. Enzyme-linked, kinetic procedures were used to measure ALT and AST; however, the linkage enzyme was malate dehydrogenase for AST and lactate dehydrogenase for ALT in the Cobas Mira (ALT, #42375; AST, #44910; BUN, #44568; cholesterol, #44307; creatinine, #44905; glucose, #44557; total bilirubin, #42287) and Ektachem, and pyruvate oxidase for both AST and ALT in the Reflotron.

Results
The inter-assay coefficient (CV) was not available for all analytes. Between-assay precision, where available, was lower than within-run precision. Intra-assay CV for all analytes was less than 5%. The inter-assay CV was 6.5% for cholesterol on the Reflotron, and 7.6% and 11%, for creatinine and AST, respectively, on the Ektachem. Correlation coefficients were greater than or equal to 0.98 for all analytes measured. Regression analysis was performed comparing both the Reflotron and the Ektachem to the Cobas (Table 1). The Student's t-test was used to determine whether the slopes were equal or different from 1.00 using an α of 0.05 (Table 2). No significant differences were found between the Reflotron and Cobas assays for BUN, creatinine, glucose, ALT, and AST. Blood urea nitrogen, creatinine and ALT values were not significantly different when measured by the Ektachem and CONTINUED...
Comparison of Chemistry Analyzers

TABLE 1

Linear Regression Results for the Reflotron (R) and Ektachem (E) Comparison to the Cobas Mira (C)

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficient</th>
<th>Y Intercept</th>
<th>Standard Error</th>
<th>Slope</th>
<th>Standard Error</th>
<th>N*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R&amp;C</td>
<td>E&amp;C</td>
<td>R&amp;C</td>
<td>E&amp;C</td>
<td>R&amp;C</td>
<td>E&amp;C</td>
</tr>
<tr>
<td>BUN</td>
<td>0.99</td>
<td>0.98</td>
<td>2.91</td>
<td>1.20</td>
<td>4.00</td>
<td>5.68</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.99</td>
<td>0.98</td>
<td>-0.06</td>
<td>0.00</td>
<td>0.20</td>
<td>0.37</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.98</td>
<td>0.98</td>
<td>6.57</td>
<td>16.84</td>
<td>9.90</td>
<td>8.19</td>
</tr>
<tr>
<td>ALT</td>
<td>0.99</td>
<td>0.98</td>
<td>3.69</td>
<td>11.91</td>
<td>5.65</td>
<td>6.19</td>
</tr>
<tr>
<td>AST</td>
<td>0.98</td>
<td>0.98</td>
<td>15.83</td>
<td>-55.60</td>
<td>59.65</td>
<td>80.47</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.99</td>
<td>0.99</td>
<td>40.64</td>
<td>26.57</td>
<td>9.90</td>
<td>8.19</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.98</td>
<td>0.99</td>
<td>-0.24</td>
<td>-0.22</td>
<td>0.57</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*N equals number of specimens

Cobas (Fig. 1). Slopes were significantly different from 1.00 for cholesterol and bilirubin for both analyzers (Fig. 2). In addition, the Ektachem revealed slopes significantly greater than 1.00 for glucose and AST (Figs. 3 & 4).

Discussion

Dry clinical chemistry analyzers are intended to perform screening tests that alert the practitioner to critical abnormalities. For this purpose they must give reproducible results over the wide range of analyte values seen in clinical medicine. All analyte procedures used with the instruments in this study met that need.

The significant differences observed for some of the analytes on the dry chemistry analyzer in relation to the reference instrument occurred as a result of a proportional systematic error. This error resulted from a decreased slope (<1.00) for cholesterol measured on both dry reagent analyzers in contrast to the Cobas, and for bilirubin and glucose measured on the Ektachem in contrast to the Cobas. The bilirubin and AST data resulted in a greater slope (>1.00) when measured on the dry reagent analyzers in the Reflotron/Cobas and Ektachem/Cobas comparisons, respectively. For glucose and AST readings values obtained were 17 mg/dL lower on the Ektachem for the highest glucose value, and 452 U/L higher on the Ektachem for the highest AST value (Figs. 3 & 4). These results suggest that the accuracy of measurements obtained on the Ektachem for glucose and AST decreased significantly when high concentrations and activities, respectively, of these analytes occurred in serum. If the highest value was excluded from the regression analysis for these two analytes, the slopes were not significantly different from one, as determined by the Student's t-test; however, loss of accuracy associated with high analyte concentrations did not appear to explain the proportional systematic error for bilirubin and cholesterol in which the comparisons were also done excluding high values. Proportional systematic errors may occur due to loss of reaction linearity when high concentrations of an analyte exist in serum. This may be associated with substrate depletion that occurs during the course of the reaction at high analyte concentrations with a given method that differs from that used by the second analyzer. Finally, a second source of proportional systematic error may be experimental error resulting from multifold dilutions that are performed manually in order to obtain readings on either dry reagent analyzer, but that are performed automatically with greater precision on the reference analyzer.

Summary

Both dry clinical chemistry analyzers showed high correlations with the reference analyzer for the analytes studied. There was insufficient evidence to reject the null hypotheses of slope = 1 for BUN, creatinine, glucose, ALT, and AST on the Reflotron; and for BUN, creatinine, and ALT on the Ektachem. The significant slope differences seen for the remaining analytes were associated with a proportional systematic error in accuracy. These were observed on both dry reagent analyzers for cholesterol and bilirubin. On the Ektachem, high glucose and AST values likewise resulted in statistically significant slope differences.

For the results above normal canine reference ranges, the Reflotron yielded higher values for bilirubin and lower values for cholesterol. The Ektachem yielded higher val-

TABLE 2

Results of Slope Analysis Using Student t-test; Null Hypotheses (H): Slope = 1

<table>
<thead>
<tr>
<th></th>
<th>t Value</th>
<th>Reflotron/Cobas T.S.</th>
<th>Reject H</th>
<th>Ektachem/Cobas T.S.</th>
<th>Reject H</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>2.26</td>
<td>2.17</td>
<td>no</td>
<td>1.09</td>
<td>no</td>
</tr>
<tr>
<td>Creatinine</td>
<td>2.26</td>
<td>2.00</td>
<td>no</td>
<td>1.17</td>
<td>no</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.20</td>
<td>0.50</td>
<td>no</td>
<td>-2.40</td>
<td>yes</td>
</tr>
<tr>
<td>ALT</td>
<td>2.31</td>
<td>1.00</td>
<td>no</td>
<td>-1.83</td>
<td>no</td>
</tr>
<tr>
<td>AST</td>
<td>2.20</td>
<td>-2.00</td>
<td>no</td>
<td>5.14</td>
<td>yes</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.31</td>
<td>-11.00</td>
<td>yes</td>
<td>-6.00</td>
<td>yes</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>2.31</td>
<td>2.42</td>
<td>yes</td>
<td>-7.50</td>
<td>yes</td>
</tr>
</tbody>
</table>

*p<0.05
*Test statistic
ues for AST and lower values for glucose, bilirubin, and cholesterol.

Loss of accuracy is to be expected with very high values outside the linear range for the assay, particularly when dilutions are performed in order to obtain a reading on the instrument; however, the differences seen in the high ranges for these analytes would not necessarily indicate a difference in the clinicopathological interpretation of the patient's status. Given the small error seen, the low maintenance requirement, the low reagent cost and ease of use, we considered the Reflotron to be more appropriate for our use outside regular hospital hours by nontechnical staff.

REFERENCES

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