Evaluation of a Reconstituted Dried Serum 
as a Clinical Chemistry Standard 
in the Abnormal Range

Bernard Klein and Milton Weissman

A lyophilized serum chemistry reference standard containing constituents in the “abnormal” range has been evaluated for use as a proficiency standard. The findings indicate that it is a satisfactory material for such use and as a reference standard, provided that it is used in accordance with the manufacturer’s directions. Interferences contributing to inaccurate results in the analysis of bilirubin, cholesterol, and uric acid are indicated and discussed. It is recommended that the standard be used for the preparation of calibration curves for bilirubin and uric acid.

REFERENCE LABORATORIES are consulting laboratories in the Veterans Administration medical program (1). In addition to their function of providing technical consultation for field-station laboratories in their assigned areas, they are charged with the periodic evaluation of the technical proficiency of the field-station laboratories.

For many years the Biochemistry section of this Reference Laboratory (Bronx) has been concerned with the examination of commercially available materials to determine their suitability as standards for proficiency evaluations. In 1958, a report from this laboratory (2), based on a collaborative study, indicated that a dialyzed dried serum (Versatol*) having constituents in the normal or physiologic range, was satisfactory as a reference standard, when

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*Versatol is a registered trade mark of the General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N. J.
properly reconstituted. This preparation has served for several years as a proficiency standard. Now that a number of "abnormal" clinical chemistry control sera have been marketed, the present report offers an evaluation of one, a dialyzed, dried serum (Versatol-A), which contains constituents in the pathologic or physiologic abnormal range.

**Materials and Procedure**

The control serum is prepared as described in the earlier report (2), except that the constituents are restored to the serum in amounts other than the normal concentrations. Stability studies of the dried material and the reconstituted serum samples were not undertaken, since this aspect was examined thoroughly in the earlier study (2). The reconstituted samples were analyzed at frequent intervals during a 6-mo. period by procedures in use in this laboratory, both by the authors and by members of the Biochemistry section (3–12).

**Results**

The results of the analyses for the indicated constituents are given in Table 1. With the exception of bilirubin, free cholesterol, and creatinine, the results came within the manufacturer's recommended 5 per cent variation. Only bilirubin fell outside the usually accepted 3 standard deviations.

**Discussion**

In this evaluation, both well-known methods and procedures not widely used were examined for sensitivity and reliability. In the latter category are the semiautomated Cotlove coulometric procedure for chloride (13) and the Technicon Autoanalyzer methods for glucose and urea (14).* From this study some evidence was obtained that, although a specified procedure may be reliable at both normal and abnormal levels, when applied to solutions of the pure substance, this may not hold for the same substance in serum, where there will be greater interplay of interferences. This topic will be considered more fully when the substances subject to interference are considered.

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*The evaluation was confined to these two determinations, although methods for calcium, chloride, phosphate, and the like have been adapted to automatic operation.
Table 1. Analysis of Lyophilized Reconstituted Abnormal Serum*

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Manufacturer's Value</th>
<th>Mean Value</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%†</td>
<td>5%‡</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>5.3</td>
<td>4.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>7.0</td>
<td>7.0†</td>
<td>0.1</td>
</tr>
<tr>
<td>Chloride</td>
<td>96.0</td>
<td>90.0††</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>89.0</td>
<td></td>
<td>1.4</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>95</td>
<td>95</td>
<td>5.0</td>
</tr>
<tr>
<td>Cholesterol (free)</td>
<td>18</td>
<td>13</td>
<td>1.6</td>
</tr>
<tr>
<td>Creatinine</td>
<td>4.0</td>
<td>4.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>205§</td>
<td>197†**</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>197**††</td>
<td></td>
<td>5.7</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>8.0</td>
<td>8.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Potassium</td>
<td>6.9</td>
<td>7.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Protein (total)</td>
<td>4.7</td>
<td>4.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium</td>
<td>122</td>
<td>123</td>
<td>1.4</td>
</tr>
<tr>
<td>Urea N</td>
<td>30</td>
<td>29</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28**††</td>
<td>0.9</td>
</tr>
<tr>
<td>Uric acid</td>
<td>8.0</td>
<td>7.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Lot No. 15978.
†Milligrams or grams per 100 ml., or millimoles per liter, as applicable.
‡Manufacturer’s recommended permissible variation.
§Reference 8.
||Cotlove et al. (13).
¶Nelson-Somogyi method (4).
**Reconstituted in 5% sodium fluoride to ensure maximal stability; see Klein, B., and Weissman, M. Clin. Chem. 4, 420 (1958).
††Reference 6.

Cholesterol

Careful attention was paid to the analyses for this constituent, since its accurate determination has assumed increased significance. Furthermore, the need for accurate control of this determination was implied in a recent article by Rivin and associates (15), who found as much as 100 per cent variation in results of cholesterol analyses by five laboratories, each staffed by qualified analysts.

Occasionally during this evaluation, without evident variation of technic, values 30–60 per cent higher were obtained by the authors. Similar experiences with identical samples were reported by the laboratories within this Reference Laboratory’s jurisdiction.

The possibility that the limited solubility of cholesterol in the reconstituted solution and improper mixing produced local differences in concentration was explored. Careful examination of the fluid disclosed no particulate matter. The interference was
finally traced to the large amounts of bilirubin present in the sample. Such interference in cholesterol analysis has been discussed by several investigators, especially in connection with the ferric chloride procedures originally introduced by Zlatkis and associates (16). Several modifications designed to overcome this interference have been introduced, involving extractions of cholesterol from serum (17-19), removal of the bilirubin by coprecipitation with the proteins (20-22), or introduction of a correction factor (23).

Comparatively little attention has been paid to bilirubin interference with the Liebermann-Burchard color development. It will be recalled that in most reports of recovery studies in which this reagent was applied to residues of organic solvent extracts, clear, unhemolyzed, nonicteric serum pools were used. In practice, it has been common experience to observe colored extracts of icteric seraums, as well as yellow protein precipitates. Bilirubin is mostly bound to albumin and is usually coprecipitated during the solvent extraction. Some proportion is extracted by alcohol, nevertheless, especially in the case of highly icteric seraums. Experiments in progress in this laboratory indicate that quantities of bilirubin equivalent to 10 mg./100 ml. give colors with the Liebermann-Burchard reagent equivalent to about 230 mg. of cholesterol. This, in turn, is equivalent to a 105 per cent increase in the average cholesterol value. A similar finding is reported by Gray (24). It is doubtless significant that the Abell procedure (25) consistently gave results within 1 standard deviation of the mean value. Here, extraction of cholesterol from serum by petroleum ether without simultaneous extraction of pigment reduces the interference from this source to a minimum.

Uric Acid

The mean value obtained in this evaluation was 7.5 mg. The mean value obtained by other laboratories in this Reference Laboratory area, using the same material, was 7.3 ± 0.6 mg. The latter also used procedures involving photometric estimation of the chromogen produced by treatment of deproteinized filtrates with urea-cyanide-carbonate and arsenophosphotungstic or phosphotungstic acid, or variations of these basic technics.

These data illustrate the interference caused by coprecipitation of the uric acid with protein (25). Repeated studies in this labora-
tory have demonstrated that while better than 95 per cent recovery can be obtained consistently by Caraway's method (10), following the addition of uric acid to deproteinized (tungstic acid) filtrates, a maximum of only 85 per cent recovery is realized by the addition of uric acid to serum. The effect of coprecipitation or adsorption of uric acid on the protein precipitate is well known, as is the chromogenic effect of other substances normally present in serum (26-28). Procedures designed to reduce or eliminate these interferences, as well as to increase the specificity, have included: use of specific enzyme action (27, 29-31) (only the more recent papers are cited); alcalinization of the serum prior to deproteinization (32, 33) and use of phosphotungstic acid as protein precipitant, with glycerol-silicate reagent and polyanethol as protective colloid to avoid turbidity (32, 33) and variations (34); and adsorption onto, and displacement development (elution) from, a Dowex-2X (Cl\textsuperscript{-}) anion-exchange resin column (35, 36). Yet a survey of uric acid methods used generally in this Reference Laboratory area revealed that most laboratories still use the classic Folin or Benedict procedures or more recent variants. It is assumed that these methods have appeal because they are simple and convenient and require few additional reagents, in that a single deproteinization provides material for analysis of uric acid as well as other substances and in that the method lends itself to the analysis of many specimens.

It is in this situation that a control serum of the type under evaluation is useful. A reconstituted control sample containing a definite amount of uric acid is used either directly as the standard against which an unknown sample is compared or to establish a calibration curve. Identity of handling both the control standard and the unknown sample reduces extraneous error and thereby gives a more meaningful result when the two values are compared.

**Bilirubin**

The analysis of the reconstituted serum for this substance again offered an interesting example of the effect of interference. The mean value obtained in this laboratory was 4.8 ± 0.1 (Table 1). The mean value obtained by the laboratories in the Reference Laboratory area was 4.7 ± 0.4. It was suspected that the lower values obtained were related to a difference in the protein present. This had been demonstrated by Seligson (37), who found that the peak absorption of diazotized bilirubin in serum was at 535 m\textmu, while azobilirubin
solutions in chloroform-methanol showed a peak at 560 m\(\mu\). Extinctions were also pH-dependent. Moreover, both the absorption peak and the extinction were found to be dependent on albumin concentration.

Recently, Lathe and Ruthven (38) examined the effects of variation of each of the ingredients of the diazotized sulfanilic acid reagent and showed that coupling can be accelerated or retarded; indirect (unconjugated) bilirubin can react in aqueous solution, thus giving elevated direct values. Meites and Hogg (39), in addition to an examination of the effect of varying the composition of the diazo reagent, made a study of the effect of albumin on the coupling reaction. They found no significant decrease in azobilirubin values when albumin was added to the pure bilirubin solution, but a small though significant decrease occurred when albumin was added to serum containing bilirubin.

These findings lead to the conclusion that calibration curves for bilirubin estimation should be prepared with solutions of bilirubin containing protein (serum). A similar suggestion was made by Seligson (37) and is supported by the present authors. The reconstituted serum control described in this report, diluted appropriately, provides a convenient material for this purpose. Such calibration procedures are now in use in the authors’ laboratory.

References
3. Technical Methods Manual. Biochemistry Laboratory, Veterans Administration Hospital, Bronx, N. Y.
Vol. 7, No. 2, 1961

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24. Gray, T. Lab Notes, Lebherbridge Memorial Hospital and St. Michael General Hospital (Montreal, Canada), 1, 24 (1959).