A Candidate Reference Method for Uric Acid in Serum. II. Interlaboratory Testing

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We describe the interlaboratory testing of a candidate Reference Method (Part I) for uric acid in serum. The method is based on the ultraviolet spectrophotometric quantitation of uric acid before and after incubation with uricase. A comprehensive investigation involving 12 laboratories was organized to document the transferability, intra- and interlaboratory precision, and general reliability of the candidate Reference Method. The interlaboratory CV with this method was about 2 to 6% for uric acid concentrations ranging from 0.12 to 0.60 mmol/L. The results detailed here demonstrate that the method can be successfully duplicated among different laboratories.

The development and evaluation of a candidate Reference Method for uric acid in serum has been previously described (1, 2). The Uric Acid Study Group of the AACC Committee on Standards16 undertook a multi-stage program to test the interlaboratory transferability of the method. A detailed protocol (1) was designed which included: (a) equipment and chemical specifications; (b) reagent and standard preparation; (c) adequacy (stability, concentration, etc.) tests for reagents and standards; (d) purity tests for reagents; (e) calibration of pipetting devices; (f) detailed instructions for the analytical procedure; and (g) guidelines for validation of the standard curve and analytical results. The specifications for equipment and reagents were based on performance criteria, so that various devices and materials in individual laboratories could be used. Preparation and distribution of serum pools, calculation of results, and other coordinating functions were carried out by the Clinical Chemistry Division, Centers for Disease Control (CDC).

Materials and Methods

Apparatus17

Spectrophotometers used by the participating laboratories were: Beckman 25, ACTA CII, Beckman UV 5260, ACTA CIII (Beckman Instrument Co., Fullerton, CA 92634), Cary 14, Cary 16, Cary 18, Technicon 635, Cary 118C (Varian Instrument Division, Palo Alto, CA 94303), Gilford 300 N (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074), and Coleman 124 (Coleman Instruments, Oakbrook, IL 60521). Automated pipetting devices used were the Micromedic Model No. 2500 (Micromedic Systems, Inc., Philadelphia, PA 19105) and the aca pipettor (DuPont Co., Wilmington, DE 19898). Centrifuges used were: IEC HNS, IEC SBV, IEC PR6000, IEC CS, IEC K&V, IEC PR-2 (Damon/IEC Division, Needham Heights, MA 02194), Sorvall GLC-2B, Sorvall RC-3 (Ivan Sorvall Inc., Newton, CT 06470), Beckman T-J6, and the Beckman J-21B.

Method

The candidate Reference Method for uric acid in serum is a differential spectrophotometric uricase procedure (2). This method was performed by the 12 participating laboratories exactly as described in the detailed protocol (1), which is available on request.

Interlaboratory Testing of the Candidate Reference Method

A multilaboratory investigation was organized to document the candidate Reference Method's transferability (i.e., intra- and interlaboratory precision, and general reliability). Twelve members of the Uric Acid Study Group agreed to set up the method in their laboratories and participate in the project. Three preliminary analytical runs (unpublished, but available on request) were done to familiarize the participants with the method protocol, standardization, instrumentation (spectrophotometers, centrifuges, automated dilutors), glassware, and reagent preparation. Special bovine-based serum pools were prepared at CDC with various uric acid concentrations and distributed to the participants in frozen aliquots. In the fourth and final interlaboratory trial, a sufficient number of samples were sent to permit each laboratory to complete four separate analytical runs, in duplicate (eight total analyses per sample), on a set of seven uric acid pools. The uric acid content in two of the seven pools was revealed to the participants before completion of the assay, and these served as internal controls. A total of 96 results were available for statistical evaluation of each pool. Each laboratory submitted original

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2 Veterans Administration Medical Center, San Diego, CA 92161.
3 Hartford Hospital, Hartford, CT 06115.
4 University Hospital, Madison, WI 53706.
5 Peninsula General Hospital, Salisbury, MD 21801 (current).
6 DuPont Co., Wilmington, DE 19898.
7 Eastman Kodak Co., Rochester, NY 14609.
8 Jewish Hospital, St. Louis, MO 63110.
9 Baker Instruments (formerly a part of Union Carbide Corp.), Rye, NY 10580.
10 Beckman Instruments Inc., Fullerton, CA 92634.
12 Food and Drug Administration, Washington, DC 20910.
13 New York State Department of Health, Albany, NY 12201.
14 Hahnemann Medical College, Philadelphia, PA 19102 (current).
15 Institute of Health Research, San Francisco, CA 94115.
16 This document has been reviewed and accepted by the AACC Committee on Standards.
17 Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Received April 10, 1981; accepted Nov. 10, 1981.
absorbance data to CDC, where all calculations were done by
the linear least-squares technique.

Results
Table 1 and Figure 1 summarize the results submitted by
each of the 12 laboratories participating in the transferability
study of the candidate Reference Method. For unknowns
having a uric acid concentration of approximately 0.14
mmol/L, 71% of the individual CVs were <5%. For unknowns
having uric acid concentrations of 0.22 to 0.33 mmol/L, 97%
of the individual laboratory CVs were <5%. Figure 1 illustrates
the individual ranges reported for two samples; ranges for the
other samples were similar. The results from a nested,
three-way analysis of variance for data submitted by all 12
laboratories (Table 2) indicates that the within-day within-
laboratory, among-day within-laboratory, and among-
laboratory variance components are essentially the same for all
samples. The overall CVs are <4% except for the two samples
with the lowest uric acid concentration, for which they are 6.8
and 5.6%.

Discussion
The intra- and interlaboratory precision were used as crit-
eria in assessing the transferability of the method. This in-
vestigation, proceeding over a period of several years, has
documented acceptable precision both within and among
laboratories and indicates that this method is transferable.

During the three preliminary trials there were numerous
changes and refinements in the protocol as the participating
laboratories encountered problems with instrumentation or
materials. Resolution of these problems yielded a candidate
Reference Method for the final trial that was robust and re-
producible in the hands of 12 different laboratory groups.
Overall precision, as measured by the average CV for each
serum pool, ranged from of 2 to 6% in the physiological range
of serum uric acid.

This study documents transferability, which is only one
phase of reference method development. The initial phase in
the development of a Reference Method involves the opti-
mization of reaction variables, documentation of recovery,
interferences, and precision, and the development of purity
criteria for reagents. These matters are reported elsewhere
for the candidate uric acid Reference Method (1, 2). Another
phase of Reference Method development is to compare results
with the isotope dilution/mass spectrometry method. The uric

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Table 1. Summary of Individual Laboratory Results: * Mean mmol/L (and CV, %)

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* Each laboratory reported eight results per sample (total = 96), except for Sample 2, for which two laboratories reported only seven results each (total = 94); Sample 5, for which one laboratory reported only seven results (total = 95); and Sample 7, for which one laboratory did not report results (total = 88). * Samples 1 and 7 are Controls 1 and 2, respectively; Samples 2–6 are unknowns.
Table 2. Summary of Nested, Three-Way Analysis of Variance * for Interlaboratory Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean, mmol/L</th>
<th>Within-day, SD (CV, %)</th>
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<th>Overall, SD (CV, %)</th>
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* Variance components were calculated for single measurements from duplicate vials on four different days, made in 12 laboratories. Degrees of freedom for sum of squares for within-day, within-laboratory; among-day, within-laboratory; among-laboratories; and overall, respectively, were: Samples 1, 3, 4, and 6—48, 36, 11, 95; Samples 5–47, 36, 11, 94; Samples 2–46, 36, 11, 93; Samples 7–44, 33, 10, 87. * Mean of 96 analyses for Samples 1, 3, 4, and 6; 94 analyses for Sample 2; 95 analyses for Sample 5; and 88 analyses for Sample 7.

Acid serum pools used in the interlaboratory transferability study have been supplied to the National Bureau of Standards for testing by an isotope dilution/mass spectrometric method developed in their laboratory. These results will be presented in a subsequent paper, and will document the accuracy of the candidate Reference Method. If adopted as a Reference Method, the procedure reported here can be useful for accuracy evaluation of new and current field methods for uric acid quantitation.

We acknowledge the assistance of Sarah McKneally and Dr. Douglas Fast in the statistical analysis of the data.

References
