Automated and Manual Direct Methods for the Determination of Blood Urea

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Automated and manual direct methods for the determination of urea in blood or serum are described. These methods determine urea by the colored product formed when urea, in relatively weak acid solution, reacts with diacetyl monoxime in the presence of thiosemicarbazide and ferric ion. Results are compared with those obtained by urease conversion of urea to ammonia and measurement of the ammonia by nesslerization.

Several modifications of the original method for the automated (AutoAnalyzer*) determination of blood urea by a direct reaction have been proposed (1, 2). To obtain adequate sensitivity for the direct reaction between diacetyl monoxime and urea, a high sulfuric acid strength (67% v/v) and an ion having oxidative capacity have been required. Although ferric ion replaces the potentially toxic arsenic pentoxide as the oxidant in the Fearon (3) reaction, the need for strong acid has remained. The disposal of such strongly acid residues has been a problem.

A method which does not call for such corrosive reagents has been proposed. The Berthelot phenate-hypochlorite reaction (4) has been adapted for the automated determination of ammonia (5, 6) and for the automated determination of ammonia from blood urea after treatment with urease (7). The method using urease is not very suitable for automated analysis since an incubation time of about 20 min. is required for the conversion of urea into ammonia.

Recently, however, thiosemicarbazide has been demonstrated to alter and intensify the color of the direct reaction between diacetyl monoxime and urea and has been used in a manual procedure for urea determination (8), and in an automated procedure requiring strong acid (9).

The present report presents automated and manual methods for the
direct determination of urea which take advantage of the increased sensitivity gained by combined use of ferric ion and thiosemicarbazide to reduce the acid requirement to about one-tenth that originally needed.

Reagents

Reagent grade chemicals are used throughout except where indicated.

Sodium chloride 0.9% (w/v) solution

Acid reagent To 100 ml. of water add 8 ml. of concentrated H₂SO₄, 1 ml. of 85% (concentrated) orthophosphoric acid, and 1 ml. of 5% acid FeCl₃.

Acid FeCl₃ solution To 100 ml. of 5% (w/v) aqueous FeCl₃ solution add 1 ml. of concentrated H₂SO₄.

Stock diacetyl monoxime (DAM) solution 2.5% (w/v) 2,3-butane-dione-2-oxime in water.

Stock thiosemicarbazide (TSC) solution 0.25% (w/v) in water.

Working DAM-TSC solution To 20 ml. of stock DAM add 40 ml. of stock TSC and 140 ml. of water.

Color reagent (manual method) To 30 ml. of acid reagent add 20 ml. of water, 1 ml. of 2.5% DAM, and 0.25 ml. of 0.25% TSC solution.

Trichloracetic acid 10% (w/v) aqueous solution.

Urea standards 5, 10, 20, 40, 60, 100, and 150 mg./100 ml. of urea nitrogen.

Methods

A flow diagram for the automated diacetyl monoxime method for blood urea determination is shown in Fig. 1. Blood, serum, or aqueous urea standards are aspirated into the system at the rate of either 40 or 60 samples per hour and diluted with 0.9% NaCl solution. This mixture is dialyzed against a 0.9% NaCl solution and the resulting dialysate is added to a mixture of DAM-TSC and acid reagent solution in a 95° glycerol bath. The pink solution resulting from this reaction is air-cooled in a mixing coil before being passed through a 10-mm. flow cell colorimeter equipped with 520-mμ interference filters. Results are calculated by comparison of data obtained for the test solutions with those obtained for standard urea solutions.

In the manual diacetyl monoxime method 1.0 ml. of water and 1.0 ml. of 10% trichloracetic acid are added to 0.2 ml. of serum. This is mixed well and centrifuged for 10 min. To 0.2 ml. of the supernatant liquid 3 ml. of the color reagent is added. After being kept in a boiling water bath for 20 min. the mixture is placed in a cold water bath to allow it to
reach room temperature. Samples and standards are read at 520 mp within 15 min.

The urease-nesslerization method is done as described by Gentzkow (10).

**Results**

**Automated Diacetyl vs. Manual Urease-Nesslerization Method**

There was an average difference of 1.4 mg./100 ml. between results of the manual urease-nesslerization method and the automated diacetyl technic when 30 blood specimens ranging in value from 6 to 114 mg./100 ml. of urea nitrogen were analyzed. The standard error of the estimate was 1.8 mg./100 ml. Calculation of the t value indicated no significant difference between these methods at the 95% probability level. The standard deviation for 45 samples analyzed in duplicate with the automated diacetyl method was 1.1 mg./100 ml. For the duplicate analysis of 35 specimens by the urease-nesslerization technic the S.D. was 1.1 mg./100 ml. Recovery experiments for the automated diacetyl method yielded values which ranged from 98 to 105% with an average of 101%.

**Automated vs. Manual Diacetyl Method**

The average difference between values obtained with the automated method and the manual diacetyl method was 1.7 mg./100 ml. for 26 serums

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**Fig. 1.** Flow diagram of diacetyl urea method.
which ranged in value from 2 to 129 mg./100 ml. of urea nitrogen. Comparison of these methods yielded a standard error of the estimate of 1.9 mg./100 ml. The paired \( t \) value determined for these methods indicated no significant difference between these methods at the 95\% probability level. The S.D. for 19 duplicate determinations by the manual diacetyl method was 1.0 mg./100 ml.

**Discussion**

Reaction conditions were selected to give a maximum yield of colored reaction product with a minimum concentration of reagents. The particular combination of sulfuric and phosphoric acids selected appeared the most effective mixture for an over-all acid concentration of 9% (v/v). The order of effectiveness of the acids tested for increasing color yield was \( H_2PO_4 > H_2SO_4 > HCl > HNO_3 \).

The color intensity of the product formed was proportional to the amount of acid and DAM used. For any particular DAM concentration there also appeared to be a maximum effective TSC concentration. Excessive amounts of TSC decreased product formation and caused turbidity in the reaction mixture.

The manual DAM urea method was developed to serve as an alternate “emergency” procedure when the automated apparatus was in use for other determinations. Similar reagents are used for both manual and automated urea determination methods.

**References**

2. Technicon Laboratory File No. 1a (1962). Technicon Instruments Corp., Chauncey, N. Y.