BEDSIDE BLOOD UREA

A STANDARD METHOD ADAPTED FOR SPEED AND SIMPLICITY

BY

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Automation brings a certain inflexibility to the routine of a clinical laboratory, but this disadvantage can be overcome by the development of rapid manual methods for use when results are required urgently (Tárnoky, 1961). It is the purpose of this paper to describe a simplified urease method for blood urea which is independent of heavy laboratory apparatus and yields results of sufficient accuracy for emergency laboratory work or for clinic and domiciliary use.

Principle.—A measured quantity of blood or serum is applied to a strip of filter paper impregnated with urease. After a short reaction period at room temperature the strip is placed in Nessler's solution containing an added protein precipitant and stabilizer. Ammonia formed from the degraded urea reacts with Nessler's solution to form a brown colour, which is compared with glass standards in a Lovibond comparator.

Apparatus

Lovibond comparator, with standard blood-urea disks, costing about £7 with a set of nine standards, or about £11 with the full range of 18 standards. While most laboratories already possess one, those who have to buy one for themselves and who need the test only as a very rough screening method might be well advised to use instead the Lovibond block comparator, which has four standards and costs £3 18s. 6d. The Tintometer Co., Salisbury, has this instrument available with standards equivalent to 36, 50, 80, and 110 mg. of urea per 100 ml. (no correction factor needed). These values are chosen as giving two standards on either side of the upper limit of normal (for greater ease of matching) plus two high values within the accurate range of the method.

Test-tube, about 3 in. (7.5 cm.) in length, to fit the sample slot of the comparator.

Pipettes: one graduated at 2 ml. and one at 0.2 ml., for reagents. One of 20 c.mm. for the specimen.

Reagents

Urease-impregnated Strips.—A strong suspension of urease is made by grinding urease-Dunning tablets† in distilled water in a small tissue-grinder. Diligence at this stage will be rewarded by a highly active preparation, since activity apparently varies inversely with particle size. The final volume should be about 0.5 ml. for every 25-mg. tablet taken, and the appearance of the suspension should be milky, with no visible particles. Pieces measuring 7.5 by 12 cm. are cut from a roll of Whatman No. 4 chromatography paper. A pencil line is drawn parallel to the longer axis of each piece, 1.5 cm. from the edge which is to be impregnated. Then 3 ml. of the urease suspension is poured as a long thin puddle into a flat 6-in. (15-cm.) Petri dish and the long edge of the paper is wetted in the urease up to the pencil line. Each face of the paper is dipped in turn and should

carry away a certain amount of free fluid on its surface; 3 ml. of suspension is sufficient for five or six pieces of paper. After dipping, the papers are rapidly dried, preferably in a vacuum desiccator. When dry, the papers are flattened between the pages of a book for a few hours and then cut at right angles to their long axes so as to provide a number of strips measuring 7.5 by 1 cm., each having a pencil line 1.5 cm. from one end to mark the effective boundary of the "active" area.

Each batch of strips should be tested for activity before use. A solution containing 200 mg. of urea per 100 ml. is treated as in the method below, but with the room-temperature reaction period varying from two to four minutes. The time which gives the greatest final colour development is used for all the remaining strips of the batch. Longer times give progressively smaller colour production (see below). The strips are conveniently stored in 25-ml. wide-neck "universal containers." The activity areas of the strips should not be touched with the fingers.

Gum Acacia (0.2%).—Two grammes of gum acacia are tied loosely in gauze. The packet is suspended overnight in 1 litre of distilled water. The next day the gauze and its remaining contents are discarded.

Stabilizing Solution.—Twelve and a half grammes of anhydrous sodium sulphate are dissolved in 50 ml. of 0.2% gum acacia. The volume is made up to 100 ml. with distilled water.

Nessler's reagent, made by any of the standard methods. (For a suitable preparation see Varley, 1958.)

Method

Blood or serum to the amount of 20 c.mm. is blown out of a pipette so as to cover the active area of a urease-impregnated strip. The strip is allowed to stand for the optimal reaction time, usually two to three minutes (see above), after which it is dropped, wetted end first, into the test-tube containing 2 ml. of stabilizing solution and 0.2 ml. of Nessler's reagent. The tube is shaken very gently for one minute, during which time colour development takes place.

After one minute the strip is removed from the tube, which is placed in the comparator slot and matched with a glass standard. The times are not critical, but they should be observed to within about 10 seconds.

Calculation

With the method described above it is necessary to correct the reading obtained on the standard Lovibond blood-urea disks as follows: (Reading on disk -5) $\times$ 1.45 = urea mg./100 ml. in specimen.

The figure 5 represent a blank correction, while the factor 1.45 corrects for the lesser sensitivity of this method compared with that for which the glass standards were prepared. The correction factors have been decided by a process of trial and error, using blood samples of known concentration: others wishing to use the method would be wise to check these values with their own reagents before putting them into general use.

Calculation is unnecessary when using the Lovibond block comparator, which is calibrated directly for this method. It would still be wise to check reagents with a known urea solution to ensure that any error is within acceptable limits.

Results

The above method was compared with the urease-nesslerization method of Archer and Robb (1925) in a series of unselected blood or serum specimens (Table I).

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†Hynson, Westcott, and Dunning, Inc., Baltimore (obtainable in this country from Messrs. Hopkin and Williams Ltd.). Other urease preparations have given less satisfactory results.

TABLE I.—*Error of the Rapid Method Determined by Comparison with Estimations Performed by the Method of Archer and Robb (1925) or by Estimation of Aqueous Solutions of Known Concentration*

Urea Concentration	No. of Tests	Error			
		mg./100 ml.		Percentage	
		Mean	Range	Mean	Range
<40 mg./100 ml. . .	37	±3.9	-8 to +9	±14.6	-28.5 to +25.8
41-70 mg./100 ml. . .	28	±4.5	-6 „ +13	±8.5	-7.6 „ +27
71-100 mg./100 ml. . .	17	±3.8	-10 „ +4	±4.6	-13.3 „ +5.6
>100 mg./100 ml. . .	14	-28	0 „ -129	-12.6	0 „ -31.3

In 60 estimations of blood-urea levels up to 120 mg./100 ml. all but one were accurate to within 10 mg./100 ml., and 75% of the results were within 5 mg./100 ml. of the correct figure. Estimations performed on aqueous urea solutions of known concentration gave results of similar accuracy.

With urea contents of above 120 mg./100 ml. the results obtained fell short of the expected values by a variable amount (Table II). When a test solution of

TABLE II.—*Individual Results at Urea Levels Over 120 mg./100 ml.*

True Value	Rapid Method	Error	
		mg./100 ml.	Percentage
196	176	-20	-10
120	113	-7	-5.8
143	143	0	0
286	206	-80	-28
222	188	-34	-15.3
125	114	-11	-8.8
154	143	-11	-7.1
128	106	-22	-17.2
412	283	-129	-31.3
232	210	-22	-9.5
150	119	-31	-20.6

200 mg./100 ml. was treated with varying reaction times the maximum reading obtained was 160-170 mg./100 ml. after two to three minutes, but this reading gradually fell to about 130 mg./100 ml. when the reaction period was extended to 10 minutes. A possible explanation of this phenomenon is that ammonia formed from the urea diffuses off the strip into the atmosphere. The urease reaction slows down as it nears completion, and the rate of loss of ammonia then overtakes the rate of its formation. Attempts have been made to control this supposed loss of ammonia by incorporating buffers into the paper strip but with little success, the buffer salts often slowing down the rate of urease reaction. "Amberlite" ion-exchange papers have been used as the base for the strips; but they are not sufficiently absorbent of fluid when impregnated with urease. Whatman cellulosic ion-exchange papers might be more suitable, but the defect does not seem of sufficient importance to justify the added cost of these modified papers.

Stability of all reagents is fairly satisfactory. Nessler's reagent prepared as in Varley (1958) tends to throw down a yellowish deposit with time but reacts satisfactorily for years rather than months. The stabilizing solution seems to give a gradually increasing blank colour over a period of six to nine months: it has been kept in a partly filled clear-glass bottle on an open shelf, and it is possible that protection from light might diminish this effect, which in any event does not significantly affect the accuracy of the method. The urease-impregnated strips have recently been tested nine months

after preparation, with satisfactory results. They have been kept at room temperature in a closed glass bottle without a desiccant.

Discussion

The very short reaction time used in this method is made possible by two features: first, the small volume in which the reaction takes place; and, secondly, the fine grinding of the urease preparation. Although urease can be obtained as a crystalline water-soluble substance, the impure and partially purified substances are particulate, much of the activity being associated with the particles. The greater the surface area exposed the greater the enzyme activity obtained. Variations of the optimal incubation time from one batch of strips to another seem to depend on variations in the original urease preparation or on the fineness with which it is ground, rather than on variations in the amount of the suspension applied to the paper.

Nessler's reagent is not very sensitive to the presence of small amounts of protein but it is strongly alkaline and will denature haemoglobin quite rapidly with the liberation of brown alkaline haematin, giving a high and variable blank. Various protein precipitants were added to the solution in an attempt to remove the haemoglobin from the reaction. Most were unsatisfactory for some reason, but sodium sulphate effectively delayed the denaturation for as long as was necessary. Precipitation of the colloidal Nessler's colour by the salt was prevented by addition of the protective colloid, gum acacia. The result is a solution with a low blank reading which, while being too turbid to read with accuracy in a photoelectric densitometer, is quite satisfactory for visual colorimetry. The high salt content probably interferes with colour development, causing the diminished sensitivity of the method compared with that of Archer and Robb (1925).

The standard urea disks for the Lovibond comparator are graduated in steps of 10 mg./100 ml., which is equivalent to more than 14 mg./100 ml. by the present method. In addition, the brown colour is a difficult one to match, especially by artificial light. (The makers specify the use of north daylight, but they can supply a "white light" screen for about £6. Alternatively, the direct light from a 60-watt "daylight blue" bulb will give satisfactory results at less cost. Fluorescent lighting is definitely unsuitable. Difficulties of this type will all affect the accuracy of the method, but hardly so much as to invalidate it as an emergency or "bedside" test, when the question asked is usually, "Is the blood urea normal, raised, or very high?"

Summary

A rapid portable method for determining the blood-urea level is described. The estimation can be completed within five minutes and the results are accurate enough for emergency work and for clinic or domiciliary use.

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