A TEST STRIP FOR THE ESTIMATION OF UREA IN SERUM

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ABSTRACT

We have developed a biostrip for determination of urea in serum. The test strip is based on enzymatic assay where urease has been immobilized on the chromatographic paper along with chromogen, phenol red. The chromogen is easily soluble in water and does not require other components for the color change. Serum urea reacts with urease and water to liberate ammonia and carbon dioxide. The liberated ammonia changes the pH of the reaction medium, which is monitored by the chromogen phenol red. A single step working reagent strip has been developed and the reaction is completed within 50 seconds at room temperature. With this test strip urea concentration is measured in serum as low as 0.15 g/L. The speed and convenience of determining urea in serum by this strip instantly makes it well suited for individuals, physicians and emergency centres.

KEY WORDS: Urea, Strip test, Urea estimation

INTRODUCTION

Test strips (dry reagent pad strips) are becoming more popular due to rapid quantitative determination of clinically relevant parameters in urine, serum, plasma and whole blood. These strips can be used in small clinical laboratories even in remote areas where sophisticated instruments are not available and also for patients' self-control. Various types of disposable test strips are available to estimate for glucose, albumin, bilirubin, urobilinogen, ketone, specific gravity, blood and pH etc. in biological fluids (1-5).

Urea is one of the first substance to be determined in biological fluids as one of the parameters of liver function tests. In routine procedures urea determination in biological fluids is carried out with chemical reagents and enzymatic methods (6-7). In literature, very few reports are available on urea test strips. The first test strip 'Urostat' for estimation of urea in plasma or serum

was reported by Tarnoky (8). The strip was made of chromatographic paper with several bands of reagents. The serum rises by capillary action and passes into a band of buffered urease.

The ammonium salt formed reacts in the next band with potassium carbonate, which liberates ammonia gas, and subsequently reacts with the next band of bromocresol green dye and changes the color in 30 minutes. In another test known as "blood urea nitrogen test" (BUN Test) a reaction slide was prepared to test urea in clinical laboratories and used in emergency (9). The test takes only 5 minutes to detect urea in serum/plasma. The slide has a dry chemical reagent pad and is divided into two chambers by a membrane. The upper one is enzymatic reaction area and bottom is detector reaction area part. Another urease reagent strip for specific detection of *Helicobacter pylori* in gastric biopsy specimens was developed by Rogge et al, (10). All these strip tests are expensive, time consuming and hence not popular in the international market. In the present study we have developed a test strip for (semiquantitative) rapid estimation of urea in serum/plasma.

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MATERIALS AND METHODS

Tris (hydroxymethyl) aminoethane, urease and β -mercaptoethanol were obtained from Sigma Chemical Co. U.S.A. and adhesive used was from Unicol, PSV. India. All other chemicals used were of analytical reagent grade.

Preparation of test strips

Urease (10mg, specific activity of 1500 U/mg protein) was dissolved in 10 ml of 25mM tris-acetate buffer, pH 5.5. To this solution 5 mg of phenol red dye was added and mixed thoroughly to make a homogeneous solution. The stability of the enzyme was increased by adding 100 μ l β -mercaptoethanol and centrifuged at 5000 rpm for 5 min to obtain a clear enzyme solution. Whatman No.1 paper (5x45cm.) was coated with enzyme dye solution and dried at 30°C in humidity free chamber. The color of the paper changes to light yellow after complete drying. The paper was cut (5 mm. width) into several pieces using a suitable cutter designed in our lab.

The high impact polymer plastic sheet (1 mm. thickness) was cut into the size of 9x90 cm. The coated piece of paper was pasted to the plastic sheet towards the length using suitable non-reactive adhesive (PSV synthetic resin adhesive diluted, 1:10 w/v with water). After complete drying, the plastic sheet, attached with paper, was cut into the size of 0.5x9.0 cm. by the cutter. Thus one end of the strip is having reactive enzymatic pad and the other one is used for handling the strip.

RESULTS

The urea strip is highly specific for detection of urea present in blood, serum and plasma. The strip gives different shades of color depending upon the concentrations of urea present in the serum samples. It ranges from light yellow to magenta color. The normal values of urea in blood/serum are 0.15-0.40 g/L and the dynamic range is 0.5-2 g/L. The color chart of urea strip was prepared and is presented in Table 1. The brown color showed normal values of urea present in serum sample whereas sera of patients showed different shades of color from light pink to magenta depending upon the concentrations of urea. The response time of this strip is 30-40 sec. The control showed light yellow color.

The test strip coated with reagent was found to be stable at room temperature for six months period. The test strip once constituted did not show any loss of enzyme activity with the time. The reaction was observed complete within 60 seconds for any concentration of urea in the tested linearity range.

The effect of different interfering substances such as glucose, ascorbic acid, amoxycillin, uric acid, hemoglobin, caffeine, gentamycin reduced glutathione and hemoglobin in the serum sample were studied with test strip. In the interference test, the concentration of each tested compound was increased up to four times, as shown in Table 2. Most of the interfering substances did not show any effect on the color formation of the strip. However, ascorbic acid showed little effect on color formation followed by amoxycillin and caffeine (Table 2).

Table 1. Estimation of urea in serum by different methods.

Concentration of urea in serum* (g/L)	Diacetyl monoxime method (g/L)	Diagnostic reagent kit** (Autoanalyzer) (g/L)	Test strip (color)
0.00	0.00	0.00	Light yellow (Control)
0.20	0.20	0.25	Light brown
0.50	0.48	0.66	Brown
1.00	0.88	1.22	Pink
1.50	1.60	1.67	Dark pink
2.00	2.07	2.20	Magenta

*The standards for serum urea was prepared by adding graded amount of urea in serum.

** M/S Bayeer Diagnostic, USA.

Table 2. Effect of different interfering substances on the strip

Substances	Concentration in mmol/L	Interference (%)
Glucose	280	0.00
Ascorbic acid	30	2.5
Amoxycillin	6.0	0.5
Uric acid	2.0	0.5
Hemoglobin	1.3	0.0
Caffeine	2.0	0.5
Gentamycin	5.0	0.0
Glutathione	0.6	0.0

Table 3. Comparative studies of biostrip and autoanalyzer.

Number of serum samples	Urea range g/L by Auto-analyzer**	Test strip color
03	0.0 - 0.20	Light brown
21	0.2 - 0.5	Brown
15	0.5 - 1.0	Pink
08	1.0 - 1.5	Dark pink
03	1.5 and more	Magenta

* 50 serum samples collected from different hospitals.

** M/S Bayer Diagnostic, USA.

For comparison studies, 50 serum samples were collected from three hospitals. These serum samples were of renal disease, diabetic, gastrointestinal hemorrhage, dehydration and normal patients.

These serum samples were first analyzed by auto analyzer (M/s Bayer diagnostic, USA) for urea concentrations. On the basis of urea concentration all serum samples were divided into five groups as shown in Table 3. The results obtained from each group were finally compared with our urea test strip. The comparative results of each group and color chart of urea strip has been presented in the Table-3. However, the color gives an idea of the range of serum urea concentration but not the exact value if the test is qualitative in nature.

DISCUSSION

The routine procedures for estimation of urea are based on o-phthalaldehyde and naphthylethylenediamine test (11), or hydrogen

peroxide measurement (12), or by auto analyzer using diagnostic reagent kit (13). These procedures are expensive, time consuming and require sophisticated instruments. Whereas, the test strip methods are simple, reliable and economical. After dipping the urea strip into the serum sample the urease converts urea into ammonia, which consequently increases the pH and changes the color of dye present in the pad of the strip. It takes only 30-40 seconds to visualize the change in color of the strip. Lower concentrations of urea (< 0.15 g/L) show very slight change in the color. Thus, whenever urea is present above the normal values (patient with kidney disease) in serum, it reacts with urease and dye to develop color, the intensity of which depends upon the concentrations of urea present in the serum. The evaluation of urea test strip was carried out by comparing the standard sera with different methods (Table1).

The effect of different interfering substances showed that if the serum contains a higher

concentration (four times higher than normal) of ascorbic acid, amoxycillin and gentamycin then there is possibility to get some false results (Table 2).

Fifty serum samples of patients were collected from different hospitals and tested in our laboratory. These results suggested that test strip has good sensitivity and specificity for identifying serum samples with urea as low as 0.15g/L (Table 3). As mentioned under results, the test is qualitative and indicates the range of serum urea concentration but not exact value.

The reproducibility and reliability of the test strip method was quite satisfactory, as compared with data obtained from enzymatic and auto analyzer methods. The effect of interfering substances does not seem to be large even with several folds increased amount of the components tested.

Test strip offers several advantages over classical enzymatic method or by auto analyzer. It is a very useful technique for qualitative as well as semiquantitative estimation of urea by doctors, patients and in small laboratories as well as in remote rural areas where lack of equipments may be the problem. The test strip method is simple, reliable and economical.

Acknowledgments

The authors are thankful to Prof. S.K. Brahmachari, Director, CBT for providing necessary facilities to carryout the work and also to the Department of Biotechnology for financial assistance.

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