Albumin test strip for quick detection of albuminuria in human

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In this article, the dry-reagent test strip technique has been discussed for qualitative and semi-quantitative estimation of albumin in urine. The strip method developed in our laboratory is quick, simple, economical and based on indigenous technique. It is based on the principle of "Protein error" in which specific chromogen immobilized onto a pad reacts with albumin present in the urine and changes the colour of the strips from light yellow to blue-green. The change in colour is visible to the naked eyes and can be compared to the colour chart for the estimation of total albumin concentration present in the urine sample.

Dry reagent test strip has become very popular for diagnosis of various diseases. Albumin synthesized in the liver, is the major plasma protein that circulates in the bloodstream. The normal concentration of albumin in blood plasma is 3.8–5.0 g/dL. Albumin transports many small molecules in the blood such as drugs, lipids, toxins, etc. It is also of prime importance in maintaining the oncotic pressure of the blood keeping the fluid from leaking out of the tissue. This is because, unlike small ions such as sodium and chloride, the concentration of albumin in the blood is greater than it is in the extracellular fluid. Low level of albumin in blood is also indication of liver diseases. Albuminuria results from kidney disease which allows albumin to escape into the urine. Albuminuria is the first sign of diabetic kidney disease.

In normal person, albumin is not excreted into the urine. Even a small amount of albumin presence in urine indicates kidney diseases. Increased level of albumin may cause major risk for diabetic patients in the face of subsequent proteinuria. Therefore, detection of albumin in urine is essential for diagnosis of albuminuria disease. Several methods have been reported for the detection of albumin in urine such as colorimetry, radioimmunoassay, immunoenzymatic assay, turbidimetry and dipstick tests. The dipsticks are marketed by “Ames” USA, in the trade name of Uristix, Multistix, Combitest, Neoestix, etc. Accurex and Span diagnosties are also marketing albumin strips for diagnosis of albumin in urine. The range of albumin test strip for urinalysis is negative to 2 g/dL.

All strips marketed in India are expensive, require more response time, cover less range and based on exogenous technology. The strips reported here, based on simple technique, are cost effective and capable to give quicker response. These strips can be handled by the patient him/herself. In this article, a simple method for the preparation of dry-reagent strip for detection of albumin in urine has been described. The technology is inexpensive and does not require expertise. The albumin strip can be used even in rural areas where laboratory testing facilities (sophisticated equipments) are not available.

Experimental Procedure

Materials

Human serum albumin (M/S Sigma chemical Co., USA), thiazole yellow G (Fluka chemicals), and bromophenol blue, citric acid and trisodium citrate purchased from S. D. Fine Chemicals. India were used. Whatman filter papers were purchased from Whatman Co. UK. Polystyrene plastic sheets and adhesive (Vamicol) were purchased from local market.

Preparation of chromogen and standard albumin solution

Bromophenol blue and thiazole yellow G (1 mg/mL each) were prepared separately in 0.1 M citrate buffer (pH 2.49). Bromophenol blue (130 μL) and thiazole-
yellow (40 µL) were mixed thoroughly with 0.9 mL of 0.1 M citrate buffer (pH 2.49) to get a homogenous solution. Stock solution (2000 mg/dL) of human serum albumin was prepared in water and dilutions (1000, 300, 100, 30 and 10 mg/dL) of albumin were made subsequently. Similarly, different dilutions of albumin were made in urine of normal person to make the colour chart and check albuminuria in human.

Preparation of dry-reagent strip

The chromogen solution (bromophenol blue plus thiazole yellow G) was immobilized on Whatman No.1 filter paper (4×30 cm) and dried at 30°C for 1 h in a humidity free chamber. After complete drying, the colour of the Whatman paper changed from white to light yellow. The paper was cut into several pieces of 5 mm width using specially designed paper cutter. The polystyrene plastic sheet (1 mm thick) was cut into 9.0×90 cm dimension. The strips of the reagent-coated pad were pasted along the edge of the plastic sheet using Vamicol (diluted 1:10 with distilled water), a non-reactive adhesive. The sheets were then dried in a humidity free chamber for 2 h. After complete drying, the sheets were cut into 0.5×9.0 cm pieces in such a manner that one end of the strip has an reagent pad and the other end is free for handling. These strips were stored in brown bottles containing silica gel bags as desiccant and stored at room temperature. Stability of the strip was monitored each week by testing the strips with standard solutions of albumin prepared in urine.

Results and Discussion

Various analytical methods developed for detection of albumin include radioimmunoassay, immunoenzymatic assay, colorimetry, turbidimetry test kit, dipstick, etc. These methods require either sophisticated equipment or trained personal. While in case of dry reagent strip reported here, an untrained person can check the albumin concentration in few seconds just by comparing the developed colour with the colour chart. The dry-reagent strip gives different shades of colour depending upon the concentration of albumin present in the urine sample. Depending on the concentration of albumin in the sample, the colour of the strip changes from yellow to bluish-green (Fig. 1). The maximum amount of albumin that can be determined by the strip is 2000 mg/dL. Control sample (without albumin) showed no change in colour of the strip. The colour of the strip does not show any change with the urine of normal person indicating the absence of albumin. However, the samples prepared by adding different amounts of albumin ranging from traces (10 mg/dL) to 2000 mg/dL in urine of normal person showed different shades of colour from yellow to bluish-green. The response time of the strip was about 20 s. Similarly, the strip was tested for urine samples of different persons to diagnose the “Albuminuria” disease (Table 1). The dry-reagent strip test is based on the “Protein error” principle of chromogens. Owing to their amphoteric nature, albumin combine with acidic as well as basic chromogens.

In dry-reagent strip, immobilized chromogens e.g. thiazole yellow and bromophenol blue react with albumin present in the sample changing the colour of the strip. The change in colour of the strip was compared with colour chart after 20 s. The efficiency of the dry-reagent strip was carried out using standard solutions of albumin in urine sample of normal person and comparing the values with other reported methods (Table 2). The results are comparable and dry-reagent
test strips developed has response time 20 s whereas dipsticks gives same colour in 60 s. The strips are stable in sealed bottle at room temperature for several months under humidity free conditions. The dry-reagent test strips are useful for qualitative as well as semi-quantitative estimation of albumin in urine sample.

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References