Critically discuss the methods that are in routine use for the measurement of albumin in serum and urine.

Albumin is the most abundant protein in serum and urine. It is a small globular protein (Mr 66 kDa) with no carbohydrate side chains, which is synthesised in the liver. Its functions include maintenance of colloidal osmotic pressure, binding and transport of substances and acting as an amino acid source, amongst others. Causes of decreased serum albumin include inflammation, hepatic disease, urinary or gastrointestinal loss or protein malnutrition. It is used in the International Staging System (ISS) for multiple myeloma. Urinary albumin is increased when glomerular permeability is increased and is therefore a measure of renal function. Use of urinary albumin/creatinine ratio (ACR) was recommended over protein/creatinine ratio (PCR) for chronic kidney disease staging in the recent KDIGO update. The use of serum and urine albumin measurements in staging protocols necessitates the use of accurate and precise methods for their measurement.

The reference method for serum albumin is nephelometry or turbidimetry. A candidate reference method using isotope dilution mass spectrometry is under investigation. A certified reference material (CRM 470) is also available. Nephelometry and turbidimetry are relatively specific methods, but are time consuming and expensive, and are therefore not routinely used in clinical laboratories. Also, the upper limit of quantification is relatively low, making these methods impractical for measurement of the high concentrations of albumin found in serum.

The most widely used methods for serum albumin are dye binding methods. The UK NEQAS scheme indicates that 60 % of participating laboratories use bromcresol green (BCG) methods, 30 % use bromcresol purple (BCP) methods and 10 % use dry slide methods (which use BCG). These methods are automatable, rapid, reproducible and cheap. The dyes have a high affinity for albumin and initial rate of binding is usually measured. However, dye binding methods are less specific than nephelometry or turbidimetry and are inaccurate if the overall serum protein pattern is abnormal, such as in multiple myeloma.

The BCG and BCP methods both have advantages and disadvantages, with neither being clearly preferable above the other. BCG shows a pronounced reactivity to various globulins, although measuring the absorbance after a short reaction time can substantially reduce the effect of non-specific binding. The BCG method underestimates albumin at high levels, and overestimates it at low levels. BCP is more specific for albumin than BCG, but is less precise due to a molar extinction coefficient with bound human albumin half that of BCG. The BCP method has been found to underestimate albumin in haemodialysis and peritoneal dialysis patients as a result of interference from the endogenous uraemic toxin 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF). This does not interfere in the BCG method. This is important as hypoalbuminaemia is used as an independent risk factor for mortality following renal transplantation. It is therefore recommended that the BCG
method be used for albumin measurement in dialysis patients. Dry slide albumin methods, which use BCG, have the advantage of using smaller sample volumes.

Other methods for measurement of albumin include electrophoresis, which can utilise cellulose acetate, agarose gel or capillary zone electrophoresis. These are semi-quantitative methods, which are expensive with a long run time. Gel methods tend to overestimate albumin since the dyes used bind albumin most avidly. Electrophoresis is therefore no longer used for quantification of albumin. There is no point of care testing method for serum albumin.

Measurement of albumin in urine is more challenging than in serum. This is due to the relatively low concentration of albumin in urine, large variation in the amount and composition of proteins present, relatively high and variable concentrations of interfering substances and the heterogeneity of albumin forms in urine. There is no reference method or certified reference material for albumin in urine and significant differences between methods have been demonstrated. To address this, an IFCC working group for the standardisation of the albumin assay in urine has been set up in collaboration with NKDEP (National Kidney Disease Education Program) and an initial report was published in 2009. Currently, most assays are standardised against the serum reference material (CRM 470), as recommended by KDIGO.

Nephelometry and turbidimetry methods are routinely used for measurement of albumin in urine in clinical laboratories. UK NEQAS reports that 5 % of participants use nephelometry, whereas 95 % use turbidimetry. Use of polyclonal antibodies in these assays allows detection of variable fragments and forms of albumin. These methods can be automated, but are relatively expensive (£2.16 quoted by NICE). High dose hook effect can be a problem, with UK NEQAS demonstrating that this phenomenon varies between analytical systems, but that approximately 10 % of laboratories may report a falsely low result in a sample containing a high albumin concentration. However, nephelometry and turbidimetry offer the sensitivity required to measure albumin at the low concentrations usually found in urine (limit of detection 2-10 mg/L).

A number of other methods can be used to measure albumin, including radioimmunoassay, enzyme-linked immunosorbent assay, size-exclusion HPLC and electrophoresis, but none of these are used routinely by clinical laboratories since they are time-consuming and expensive. A liquid chromatography tandem mass spectrometry method has been developed by the Mayo Clinic (Rochester), which measures the N-terminal 24 amino acid fragment of albumin. This is a candidate reference method, but it is not known whether the measured fragment is a constant proportion of the total urine albumin in health and disease.

“Dip-sticks” are routinely used for point of care estimation of urine albumin. These are cheap (£0.09 for Siemens Albustix) and simple to use. However, they are only semi-quantitative and do not detect albumin below 150 mg/L. Strips from different manufacturers perform differently at the cut-off concentration of 300 mg/L. False positives can occur if the urine is concentrated or if the urine is alkalinised, such as in
urinary tract infections. Interpretation is operator-dependent and is affected by the presence of coloured compounds such as bilirubin and certain drugs, but automated devices for reading the reagent strips, such as the Clinitek status, remove operator variability. NICE guidelines do not recommend the use of dip-sticks since they have poor sensitivity for disease, fail to detect some forms of kidney disease in the early stages and can give false negative results in cases where urine is dilute. However, reagent strips capable of measuring low concentrations of albumin have recently become available, such as the Clinitek microalbumin reagent strip, and the DCA 2000+ device is capable of reporting a fully quantitative albumin creatinine ratio, which may reduce some of the risks associated with previous reagent strips.

In conclusion, the inclusion of serum and urine albumin measurement in staging protocols for kidney disease and multiple myeloma, respectively, necessitates the use of accurate, precise and comparable albumin methods. Currently, serum albumin methods are better standardised than those for urine, but an IFCC working group is currently addressing the standardisation of urine albumin methods. The limitations of the methods used in each laboratory should be taken into consideration when interpreting serum and urine albumin results, and clinicians using staging systems should be aware of any bias in the methods used.