Chronic Kidney Disease: The Role of Clinical Chemists

Gary L. Horowitz, MD
Beth Israel Deaconess Medical Center
Boston, MA

Objectives

- Define Chronic Kidney Disease
- List the 4 variables in the MDRD Equation
- List the limitations of dipstick protein testing
- Define FE(Na)

National Kidney Disease Education Program

- Chronic Kidney Disease: a public health issue
- Economical, effective testing and therapies exist
- Neither testing nor therapy is adequately applied

Reminiscent of Hypertension in the 1970s

www.nkdep.nih.gov

Topics To Be Covered

- NKDEP/Chronic Kidney Disease
  - Estimated GFR (eGFR)
  - Urine Albumin (Albumin/Creatinine Ratio)
- Other Tests/Concepts
  - Cystatin C
  - NGAL (neutrophil gelatinase-associated lipocalin)
  - FE(Na)

Definition of CKD (Chronic Kidney Disease)

- Persistent GFR < 60 ml/min/1.73 m^2

and/or

- Urine albumin/creatinine ratio of > 30 mg/g (>3.4 mg/mmol)

Case History

- A primary care physician (PCP) calls you, the lab director.
- One of his patients, a 50 year old white woman with hypertension and diabetes, saw her lab results on line.
- Her serum creatinine (1.0 mg/dL (88umol/L)) was in the normal range (0.4-1.1 (35-97)), but her “estimated GFR” was low, suggesting “chronic kidney disease”.
- From her reading online, she couldn’t understand how that could be possible – Didn’t a normal creatinine mean her kidneys were fine?
- After thinking about it, the PCP agreed with her – There must be something wrong with one of the tests, and he’d like you to explain.

Definition of “Clearance”

- Clearance is that volume of plasma, per unit of time, from which a substance is completely cleared by the kidneys

- **C = UV / P**
  - U is urine concentration
  - V is volume per unit time (e.g.,2000 mL/1440 minutes)
  - P is plasma (or serum) concentration

- ***If*** a marker has the following characteristics
  - stable serum concentration
  - physiologically inert
  - freely filtered at glomerulus
  - not secreted, re-absorbed, or metabolized by kidney
- ***Then***
  - amount in urine = amount filtered at glomerulus, and
  - Clearance = GFR

Creatinine

- produced in proportion to muscle mass
- in muscle, CK catalyzes the conversion
  of creatine to phosphocreatine, a high energy compound
- creatinine forms spontaneously and irreversibly from creatine,
  at a rate of roughly 1-2% per day of the total creatine
- because it is freely filtered at the glomerulus,
  not re-absorbed or secreted by the tubules,
  its concentration is relatively constant over time
  unless there are changes in GFR
- as GFR falls, [creatinine] rises, until its concentration is high
  enough that the new filtered amount equals the amount formed

So A True Clearance Should Work, Right?

- Yes, but don't underestimate the difficulties:
  - collecting a 24-hour urine is, at a minimum, a hassle
  - fraught with inaccuracies, too:
    - GFR = C_{creat} = UV/P, where UV is total creatinine excreted
    - over-collection ➔ GFR overestimated
    - under-collection ➔ GFR underestimated
  - correct way
    - first morning X 1 (i.e., start, or end, with first morning urine)
    - gross estimate 15 mg/kg for women, 20 mg/kg for men
    - we don't get weight, so we can't know whether it's complete

Ways to Measure GFR

- Inulin Clearance – the “gold standard”
  - Requires continuous intravenous infusion
  - Plus timed urine collection
  - Measurement of inulin itself is difficult and not widely available
- I^{125}-Iothalamate Clearance
  - A single intravenous bolus suffices
  - Measurement, though it involves radioisotopes, is relatively easy
- Serum Creatinine
  - Trivial measurement, done routinely in virtually every laboratory
- Creatinine Clearance
  - Requires accurate timed urine collection
- Cystatin C
  - Not yet ready for prime time

Ways to Estimate GFR
(mathematical transformations)

- Cockroft-Gault
  - age, weight, creatinine
- “MDRD”
  - = “Modification of Diet in Renal Disease”
    - 4-parameter: age, gender, creatinine, race
    - 6-parameter: above, plus BUN & albumin

Cockroft-Gault Equation

$$GFR = \frac{(140 - \text{Age}) \times (\text{Lean Body Weight [kg]})}{72 \times P_{\text{creatinine (mg/dL)}} \times (0.85 \text{ if female})}$$

- As age increases, GFR decreases at same creatinine
- As body weight increases, at same creatinine, GFR increases
- But if body weight increase is fat (not muscle), shouldn't use equation
- This is **not** normalized to a standardized BSA
- ➔ worst case, a large overweight person whose weight we get will be deemed to have good renal function ! ! ! !

MDRD Equation
(for patients > 18)

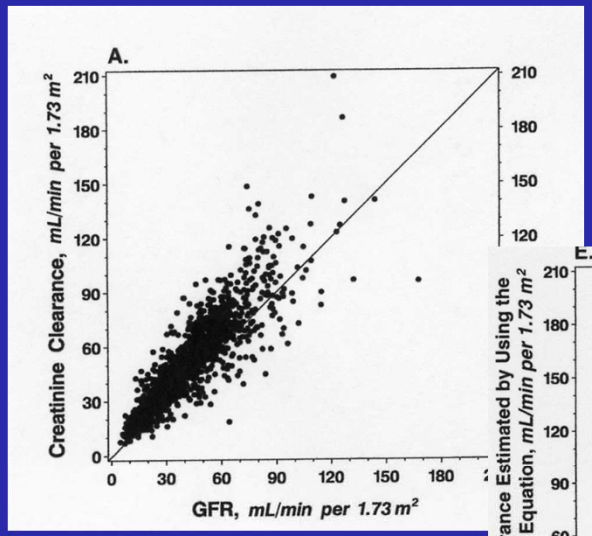
$$GFR = 175 \times (S_{creat})^{-1.154} \times (Age)^{-0.203} \times 0.742 \times 1.210$$

assumes mg/dL units
(divide umol/L by 88.4)

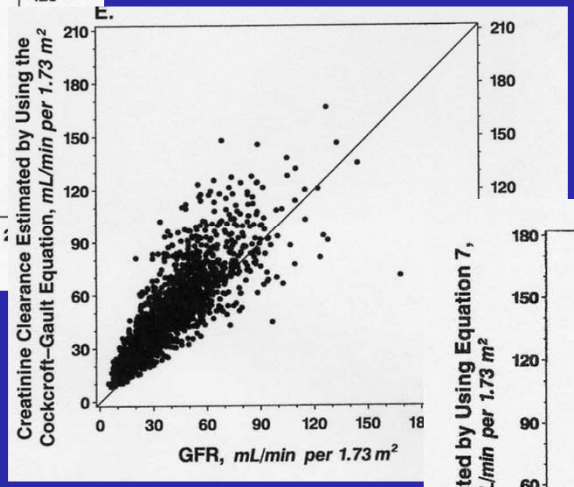
if female

if African-American

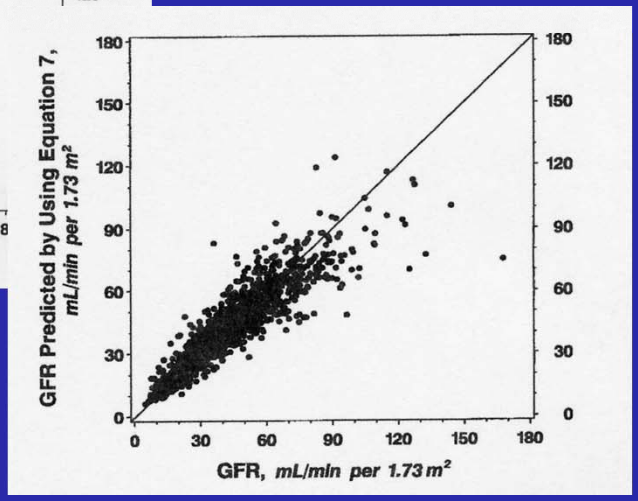
Comparison



Creatinine Clearance
(need 24-hr urine)



Cockroft-Gault
(need weight & BSA)



MDRD

Levey AS et al. Ann Intern Med 1999;130:461-470

Reporting Recommendations

- Creatinine = 1.8 mg/dL (159 umol/L)
  Estimated GFR = 30 ml/min/1.73 m^2 if non African-American
  Estimated GFR = 37 ml/min/1.73 m^2 if African-American
  Average GFR for 60-69 year old = 85 ml/min/1.73 m^2

- Don’t report values > 60 ml/min/1.73 m^2
- Don’t use for patients < 18 years old

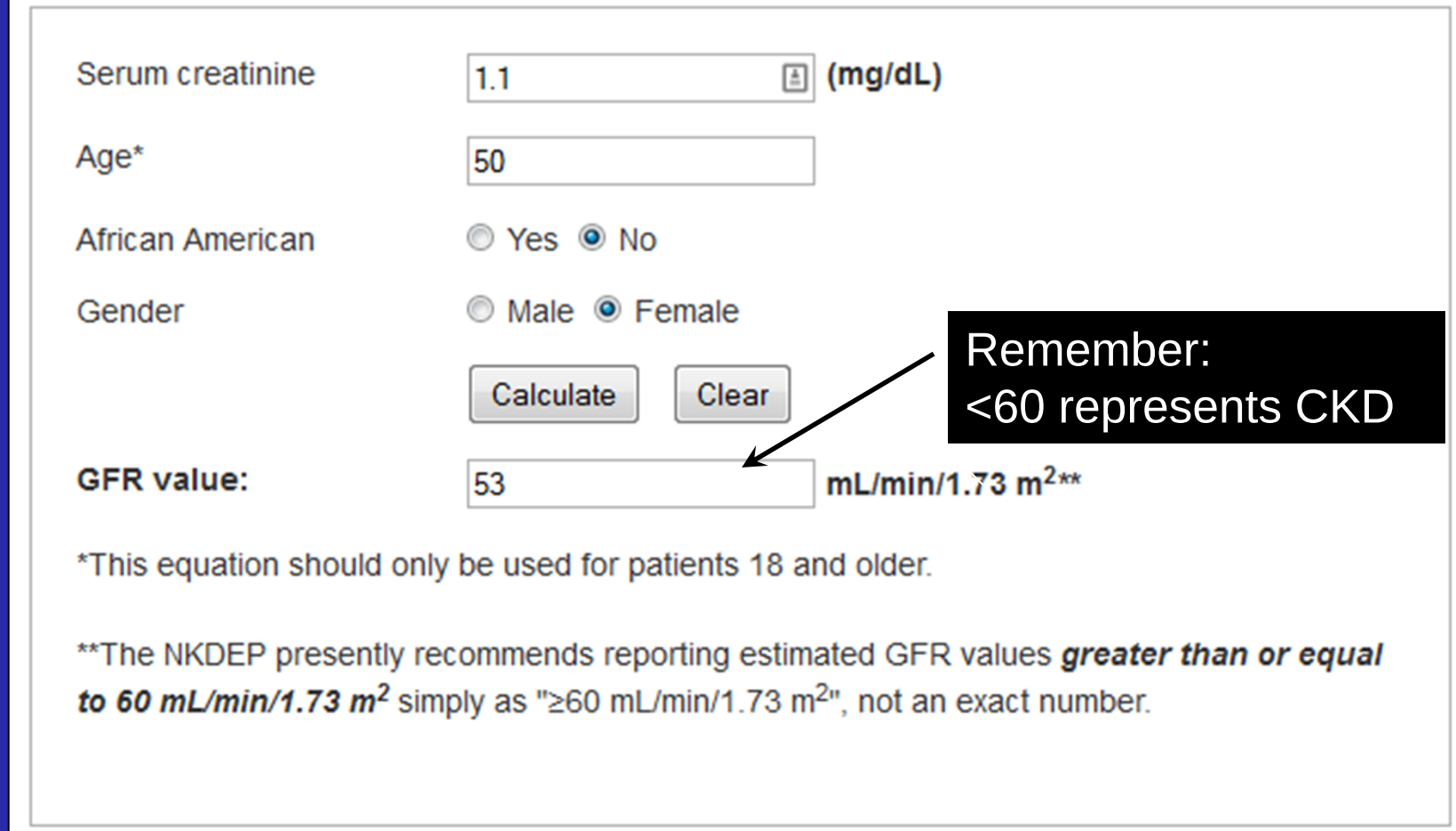
CKD-EPI Equation

www.nkdep.nih.gov

Race	Sex	Serum Creatinine, S_{cr} (mg/dL)	Equation (age in years for ≥ 18)
Black	Female	≤ 0.7	$GFR = 166 \times (S_{cr}/0.7)^{-0.329} \times (0.993)^{Age}$
Black	Female	> 0.7	$GFR = 166 \times (S_{cr}/0.7)^{-1.209} \times (0.993)^{Age}$
Black	Male	≤ 0.9	$GFR = 163 \times (S_{cr}/0.9)^{-0.411} \times (0.993)^{Age}$
Black	Male	> 0.9	$GFR = 163 \times (S_{cr}/0.9)^{-1.209} \times (0.993)^{Age}$
White or other	Female	≤ 0.7	$GFR = 144 \times (S_{cr}/0.7)^{-0.329} \times (0.993)^{Age}$
White or other	Female	> 0.7	$GFR = 144 \times (S_{cr}/0.7)^{-1.209} \times (0.993)^{Age}$
White or other	Male	≤ 0.9	$GFR = 141 \times (S_{cr}/0.9)^{-0.411} \times (0.993)^{Age}$
White or other	Male	> 0.9	$GFR = 141 \times (S_{cr}/0.9)^{-1.209} \times (0.993)^{Age}$

- uses same 4 variables as MDRD,
  but uses slightly modified equations for different combinations of those variables
- not appreciably better than MDRD for eGFR < 60
- but, if you want to report values >60, you should use it

Back to Our Case History



Remember:
<60 represents CKD

- Clearly, a creatinine of 1.1 is not “normal” for a 50 year old non-African American female
- ➔ an example of converting data into ***information***

Cystatin C

- small molecular weight protein (122 amino acids)
- produced by all nucleated cells
- filtered at glomerulus, not reabsorbed or secreted
- appears not to vary with age, sex, muscle mass
- equations for calculating eGFR exist
  - with and without creatinine
- a lot to recommend it

- standardization has been a problem
  even when the same assay is used in different labs!
- an advantage of creatinine, for screening, is that
  physicians are routinely ordering it already (no incremental cost)

Ferguson MA et al. Established and emerging markers of kidney function. Clin Chem 2012;58:680-689.

Definition of CKD (Chronic Kidney Disease)

- Persistent GFR < 60 ml/min/1.73 m^2

and/or

- Urine albumin/creatinine ratio of > 30 mg/g (>3.4 mg/mmol)

Proteinuria Physiology

- virtually all proteins are too large to be filtered through a healthy glomerulus
- once proteins do leak, there is no mechanism to reabsorb them
- urine protein concentration reflects amount leaked ***plus water content of urine*** , which varies with hydration
- provides rationale for reporting urine protein not simply as concentration but as 24º collection

Protein/Creatinine Ratio

- creatinine filtered through glomerulus
- largely unsecreted and unreabsorbed by tubules
- thus, its urine concentration reflects amount filtered
  plus water content of urine, which varies with hydration
- if you divide $[protein]_{urine}$ by $[creatinine]_{urine}$,
  since water content of urine is in denominator of both,
  you eliminate the effect of hydration status
- urine protein/creatinine ratio is an
  excellent surrogate for 24º urinary protein
  and can be done on any spot/random urine!

$[protein]_u$ is misleading

- what can happen when you rely on [protein] alone
- NB: conventional chemistry assay is no better !!

sample	dipstick protein	(estimated) dipstick mg/dL	Chemistry protein mg/dL	creatinine mg/dL	prot/ creat ratio	
1	1+	30	38			
2	1+	30	46			
3	2+	100	86			
4	3+	300	279			
5	3+	300	358			

$[protein]_u$ is misleading

- what can happen when you rely on [protein] alone
- NB: conventional chemistry assay is no better !!

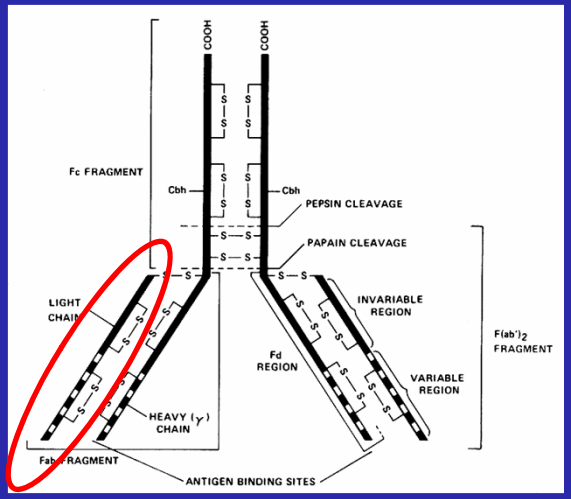
sample	dipstick protein	(estimated) dipstick mg/dL	Chemistry protein mg/dL	creatinine mg/dL	prot/ creat ratio	
1	1+	30	38	47	0.8	
2	1+	30	46	352	0.1	
3	2+	100	86	55	1.6	
4	3+	300	279	137	2.0	
5	3+	300	358	230	1.6	

False Negative Type 1

- **Bence-Jones Protein (BJP)**
  - **monoclonal free light chains**
  - **by definition, very small (23 kD)**
  - **so small, filtered by normal glomerulus (even without albuminuria!!)**

- ***not*** **detected by dipstick method**

Adapted from
Burtis, CA & Ashwood, ER.
Tietz Fundamentals of Clinical Chemistry (4th Edition).
Philadelphia: W.B Saunders, 1996, p.135.



Urine Protein Methods

- **dipstick:**
  - **method: protein error of pH indicators (c1909)**
  - **detects albumin > globulin > BJP**

- **conventional chemistry assay:**
  - **method: denature protein, then detect resulting turbidity using spectrophometry**
  - **sensitive to all proteins, including BJP**

➔ **If a sample is dipstick negative, chemistry positive, it's probably BJP**

- **(micro)albumin:**
  - **method: immunoassay**
  - **detects *only* albumin**

False Negative Type 2

- dipstick protein is not sensitive enough
  to rule out pathologic levels of proteinuria
  cannot distinguish low levels from 0

- definition:
  - analytic sensitivity = how low you can go?

- assays for which sensitivity is particularly important:
  - TSH (3rd generation)
  - CRP ("hs-CRP")
  - Troponin
  - D-Dimer
  - *and, yes, urine protein!*

Analytic Sensitivities

method	value from package insert	value in (mg/dL)	sensitivity (relative to dipstick)
Dipstick	18 mg/dL	18	
Urine Protein	6 mg/dL	6	3X more sensitive
Urine Albumin (microalbumin)	3 mg/L	0.3	>50X more sensitive
Serum Protein	0.2 g/dL	200	
Serum Albumin	0.1 g/dL	100	

Albumin

Tina-quant ® Albumin

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Calibration

Traceability: This method has been standardized against CRM 470.

Roche/Hitachi 902 analyzers

S1	0.9% NaCl
S2-6	C.f.a.s. PUC

Calibration is performed with C.f.a.s. PUC via serial dilution (6-point calibration).
Preparation of S1-6:

No.	NaCl solution (0.9%)	C.f.a.s. PUC	Assigned value conversion factor
1	200 µL	–	0.0
2	1440 µL	20 µL	0.01370
3	860 µL	20 µL	0.02273
4	420 µL	20 µL	0.04545
5	110 µL	100 µL	0.47619
6	–	200 µL	1.00000

The calculated values for the dilution series are keyed into the analyzer.

Roche/Hitachi 917/MODULAR analyzers

S1	0.9% NaCl
S2-6	C.f.a.s. PUC

Calibration is performed with C.f.a.s. PUC via serial dilution made

Calculation

The analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: mg/L x 0.0152 = µmol/L

Limitations - interference[7]

Criterion: Recovery within ± 10% of initial value.

Icterus: No significant interference up to an approximate conjugated bilirubin concentration of 66 mg/dL or 1128 µmol/L.
Hemolysis: No significant interference up to an approximate hemoglobin concentration of 300 mg/dL or 186 µmol/L.
No interference by acetone < 60 mmol/L, ascorbic acid < 5.68 mmol/L, creatinine < 44.2 mmol/L, glucose < 111 mmol/L, uric acid < 4.17 mmol/L, urea < 700 mmol/L and urobilinogen < 338 mmol/L.
Seventeen frequently used pharmaceuticals were tested in vitro. No interference with the assay was found.

With the exception of the MODULAR P antigen excess check application, a high-dose hook effect may occur at albumin concentrations above 2500 mg/L (38.0 µmol/L).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

Roche/Hitachi 902 analyzers

Measuring range:** 3–400 mg/L (0.046–6.08 µmol/L)
At higher concentrations manually dilute the sample with 0.9% NaCl (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

Roche/Hitachi 911/912/917/MODULAR analyzers

Measuring range:** 3–400 mg/L (0.046–6.08 µmol/L)
Extended measuring range with rerun:*** 3–3000 mg/L (0.046–45.6 µmol/L)

To eliminate the possibility of reporting falsely low results on specimens in excess of the Heidelberger limit (2500 mg/L), test these specimens with a urine dipstick and dilute appropriately before performing the assay. Multiply the result obtained by the appropriate dilution factor.

Microalbumin Summary

- **make sure you help clinicians order the *right test*:**
  - **for CKD screening (most sensitive), microalbumin & creatinine on random/spot urine**

- **make sure you get the *right answer*:**
  - **check the package insert for your method**
  - **if appropriate (and it can't hurt),**
    - **compare with urine total protein**
    - **run dilution on all samples, or**
    - **screen with dipstick protein**

Acute Kidney Injury (AKI)

- Currently, we depend on increases in creatinine (or cystatin C) to detect AKI (acute kidney injury)

- What if there was an AKI marker (akin to cardiac troponin) that increased before functional damage occurred?

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

- highly expressed in the tubular epithelium of the distal nephron
- released during AKI
- multiple forms in urine
- measured by ELISA

- promising, but not yet ready for “prime time”

Fassett RG et al. Biomarkers in chronic kidney disease. Kidney Int 2011;80:806-821.

Fractional Excretion Na (1)

- a calculation used to help determine the cause of AKI
- it is probably best thought of as a “ratio of ratios”:
  - **$(U_{Na}/S_{Na}) / (U_{creat}/S_{creat})$**, where
    U_X is the concentration of X in urine,
    S_Y is the concentration of Y in serum
- it tells you what proportion of the Na filtered through the kidney glomeruli is actually excreted in the urine (indicating how much of the filtered Na is reabsorbed)
- use only in patients with AKI and low urine output (oliguria)

Fractional Excretion Na (2)

- in pre-renal AKI (low blood flow to kidneys),
  - FE(Na) < 1%
  - kidneys are reabsorbing maximal amounts of Na, to try to replete volume
- in intrinsic AKI (e.g., Acute Tubular Necrosis),
  - FE(Na) > 2%
  - kidney tubules are damaged and cannot reabsorb Na
- in patients taking diuretics, FE(Na) may not be reliable because diuretics increase Na excretion
- FE(urea) can be helpful
- FE(urea) < 35% suggests pre-renal AKI

Self-Assessment Question 1

Which of the following lab tests is used in making a diagnosis of CKD?

A) serum cystatin C
B) FE(Na)
C) urine glucose
D) urine albumin/creatinine

Self-Assessment Question 2

Which of the following variables is ***not*** part of the MDRD equation for estimated GFR?

A) serum creatinine
B) age
C) gender
D) serum cystatin C

Self-Assessment Question 3

Which statement about urine dipstick protein is ***true***?

A) It will reliably detect Bence-Jones protein
B) It can be used to screen for antigen excess for immunoassays for urine albumin.
C) By itself, it can be used to give a good estimate of the severity of proteinuria
D) It is adequately sensitive to detect microalbuminuria