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A story about Creatinine Standardization!!

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Today, serum creatinine (SCr) is still one of the most prescribed analyses in medical laboratories to estimate the glomerular filtration rate (GFR). Understanding by laboratories world-wide of the importance of reliable serum creatinine measurements in GFR estimation and of factors that may affect creatinine measurement is critical for the identification of patients with CKD. Serum creatinine assays must be properly calibrated and traceable to a high-order reference system in order to eliminate or significantly reduce variation among laboratories.

Different methods to Measure Serum Creatinine: Are There any Differences between Methods? Are These Differences Significant?

Serum creatinine can be measured by the variety of methods. The basic idea behind the standardization of creatinine measurement is that all laboratories calibrate their creatinine assays against calibration material provided by manufacturers which is traceable to Gas Chromatography-isotope-dilution mass spectrometry (GC-IDMS) which is a reference method. Measurement of serum creatinine by IDMS is both accurate and very reproducible. IDMS is very expensive and cumbersome technique for the routine use. Since the Creatinine Standardization Program has requested the manufacturers to standardize their creatinine assays to IDMS reference method, considering this, we can theoretically believe that the same sample will give the same result in any laboratory in the world, whatever the method (Jaffe or enzymatic) and manufacturer, since the calibrators will all be "traceable" to the higher-order method.

Jaffe method: In 1886 Jaffe described the chemical methods for creatinine measurements are primarily based on the reaction with alkaline picrate. In this reaction creatinine reacts with picrate ion in an alkaline medium to yield an equimolar orange-red Janovski complex. In spite of considerable literature on the subject, the reaction mechanism and the structure of the product remain unclear.

In 1904, Folin described the use of this reaction to measure creatinine in urine. He later extended this principle to creatinine measurement in deproteinized blood. This reaction was nonspecific for creatinine, and a variety of adaptations have been incorporated in an attempt to remove interference from non-creatinine chromogens.

Method 1: Jaffe; spectrophotometric, end point, quantitative
Principle of analysis: Creatinine + alkaline picrate → Janovski complex (red)
Comments: Serum, plasma, diluted urine; described by Jaffe, 1886. Significant interference from non-creatinine chromogens.

End-Point Assays: This requires the use of a protein-free filtrate. Creatinine was then adsorbed onto aluminum magnesium silicate clays, including Fuller's earth (Floridin) and Lloyd's reagent, theoretically isolating it from potential interferents prior to reaction with alkaline picrate. Such techniques were extensively used in the 1970s. However, it became apparent that such methods did not

eliminate interference from non-creatinine chromogens. Further, they were imprecise and unsuitable for automation, and by 1980s they were not widely used.

Method 2: Jaffe/Fuller' s earth;; creatinine isolated before analysis; can be removed with buffer or picrate reagent added directly to creatinine adsorbent suspension
Principle of analysis: Same as Method 1
Comments: Serum, plasma, diluted urine; alternatively cation exchangers used as adsorbent. Interference from non-creatinine chromogens reduced. No longer in routine use.

Kinetic Assays : Kinetic Jaffe assays take advantage of the fact that creatinine and non-creatinine chromogens have a differential rate of color development, thus allowing a rate-dependent separation of creatinine from interfering substances. This approach significantly reduces but does not eliminate interferences from non-creatinine chromogens. Such methods gained in popularity with the advent of instruments capable of making accurate absorbance readings at precise, highly reproducible intervals. Kinetic Jaffe assays have been implemented on various automated microprocessor-controlled instruments and are currently the most widely used approach to creatinine measurement.

Method 3: Jaffe, kinetic; spectrophotometric, quantitative, kinetic analysis during early color formation
Principle of analysis: Same as Method 1
Comments: Serum, plasma, diluted urine; requires automated equipment for accurately timed and precise absorbance measurements. Precise conditions vary between instruments. Interference from non-creatinine chromogens reduced. This is currently the most popular method in diagnostic laboratories.

Enzymatic Assays: Several creatinine-degrading enzymes have been investigated for use in creatinine analysis. However, owing to the requirement for large quantities of pure enzyme, these approaches have only recently become commercially feasible. There are primarily three approaches: Creatininase, Creatininase and creatinase, Creatinine deaminase.

High-performance liquid chromatography (HPLC): Cation-exchange or reversed-phase chromatographic separation of creatinine from other compounds.
Method 4: HPLC
Principle of analysis: Creatinine quantitated by Method 1 or absorption at 230 nm or by enzymatic assay
Comments: Highly specific and precise, not used in routine practice, possible reference method.

Reference and Preferred Methods: GC-IDMS has been proposed by the Joint Committee on Traceability in Laboratory Medicine (JCTLM) as the definitive reference method for serum creatinine measurement. GC-IDMS is considered the method of choice for establishing the true concentration of creatinine in serum because of its excellent specificity and precision. Although these methods are generally used by commercial manufacturers to establish traceability of their assays, they are presently unlikely to find their way into more general use.
Method 5: Isotope-dilution mass spectrometry (IDMS)
Principle of analysis: Measurement by IDMS following either initial GC or HPLC separation.
Comments: Highly specific and precise measurement. Reference method proposed by JCTLM. HPLC-IDMS approaches may be applicable to routine use in the future.

Ref: https://www.niddk.nih.gov/health-information/professionals/clinical-tools-patient-management/kidney-disease/laboratory-evaluation/glomerular-filtration-rate/creatinine-standardization