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Creatinine Liquid Reagents Assay

Configuration

The Diazyme Creatinine Liquid Reagents assay is provided in bulk and the following kit configurations:

REF	Kit Size
DZ072B-K	R1: 1 x 60 mL R2: 1 x 20 mL Cal: 1 x 1 mL

Intended Use

Diazyme Creatinine Liquid Reagents Assay, in conjunction with Diazyme Creatinine Calibrator, is intended for the quantitative determination of creatinine in serum and urine. Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes. For *in vitro* diagnostic use only.

Clinical Significance

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2% of the body's creatine is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose of it in the urine. The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired the creatinine level in the blood will rise. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys, sometimes even before a patient reports any symptoms. It is for this reason that standard blood and urine tests routinely check the amount of creatinine in the blood.[1-2]

Assay Principle

Diazyme Creatinine Liquid Reagents Assay is a quick, easy to use enzymatic procedure applicable to routine laboratory instrumentation. Enzymatic methodology is a better clinical choice for the accurate measurement of creatinine, especially for neonates, pediatrics, and hematology units.[3] The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which itself is converted to sarcosine by creatine amidinohydrolase (creatinase), followed by oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye.[4]

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatinase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{SOD}} \text{formaldehyde} + \text{Glycine} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{Toos} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye } (\lambda \text{ max } 556)$$

Any endogenous creatine present in the sample is removed by creatinase and sarcosine oxidase during preincubation.

Reagent – "Working Solutions"

Active Ingredients	Concentration
REAGENT 1	
Creatinase	12 IU-60 IU/mL
TOOS	0.07 mg-0.21 mg/mL
SOD	4-17 IU/mL
REAGENT 2	
Creatininase	135 IU ~ 670 IU/mL
4-AA	0.3 – 0.9 mg/mL
POD	
CALIBRATOR	5 mg/dL (442 µM/L) creatinine in 0.9% saline

Precautions

- For in vitro diagnostic use only.
- Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
- As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
- Avoid ingestion and contact with skin or mucous membranes. See Safety Data Sheet.
- REAGENT 1 and REAGENT 2 contain < 0.1% sodium azide (NaN_3) as a preservative, which may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup. Reagents are light-sensitive. Do not let bottles remain open. Keep containers tightly stoppered.
- Do not use the reagents after the expiration date labeled on the outer box.
- Additional safety information concerning storage and handling of this product is provided within the Safety Data Sheet for this product. To obtain an SDS, please contact our customer service department at 858-455-4768.

Reagent Preparation

Diazyme Creatinine Liquid Reagents Assay Reagents (R1, R2 and CALIBRATOR) are ready to use, liquid REAGENT.

Reagent Stability and Storage

Unopened REAGENT materials are stable until the expiration date printed on the outer box when stored at 2–8°C. REAGENT on-board stability is at least 30 days.

Specimen Collection and Handling

Use fresh patient serum samples. Urine samples should be 10–fold diluted with saline.

Materials Provided

See "Reagents – 'Working Solutions'"

Materials Required But Not Provided

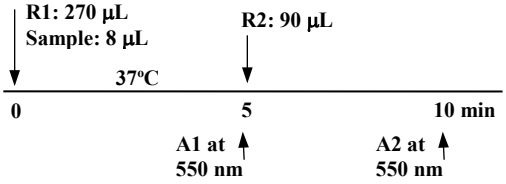
Application sheets for use of Diazyme Creatinine Liquid Reagents Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com. Controls for validating the performance of the creatinine reagents and saline for diluting urine samples are not provided.

Assay Procedure

Conditions

Please contact Diazyme Laboratories Customer Service for instrument-specific parameters.

Test Scheme for Chemistry Analyzers



Calibration

The Diazyme Creatinine Calibrator (REF DZ072B-CAL) is included with the REAGENT and, along with 0.9% saline as a CALIBRATOR 0, should be used as directed to calibrate the procedure. The CALIBRATOR is traceable to NIST SRM 967a, and should be stored at 2–8°C. Calibration should be performed at least every 14 days and with each new lot of REAGENT. Calibration is performed by entering the value as shown on the lot specific certificate of analysis.

Quality Control

We recommend that each laboratory use the Diazyme Creatinine Control (REF DZ072B-CON) to validate the performance of creatinine reagents. And is available from Diazyme Laboratories. If the results from the CONTROL fall outside the acceptable limits, as determined by the assigned range, the test should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines.

Results[5]

Creatinine concentration is expressed as mg/dL or µmol/L
mg/dL x 88.4 = µmol/L
µmol/L x 0.0113 = mg/dL

When the CALIBRATOR values are input as per instructions in µmol/L or mg/dL, the results are printed out automatically in µmol/L or mg/dL.

Muscular young or middle-aged adults may have more creatinine in their blood than the norm for the general population. Elderly persons, on the other hand, may have less creatinine in their blood than the norm. Infants have normal levels of about 0.2 mg/dL (17.7 µmol/L) or more, depending on their muscle development. A person with only one kidney may have a normal level of about 1.8 or 1.9 mg/dL (159.1 or 168.0 µmol/L). Creatinine levels that reach 2.0 mg/dL (176.8 µmol/L) or more in babies and 10.0 mg/dL (884.0 µmol/L) or more in adults may indicate the need for a dialysis machine to remove wastes from the blood.

Certain drugs can sometimes cause abnormally elevated creatinine levels. It is strongly recommended that each laboratory establish an expected range characteristic for the local population.

Reference Range[6]

	Men	Women
Serum	59-104 µmol/L	45-84 µmol/L
	0.67-1.17 mg/dL	0.51-0.95 mg/dL
Urine*	3540-24600 µmol/L	2550-20000 µmol/L
	40-278 mg/dL	29-226 mg/dL

*First morning urine

Limitations

A sample with a creatinine level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

Performance Characteristics

All performance characteristics were determined at Diazyme Laboratories using Hitachi 917.

Limit of Detection

The limit of detection is 12 µmol/L (0.14 mg/dL).

Accuracy

The performance of this assay was compared with the performance of a legally marketed creatinine assay using serum samples ranging from 0.2 – 13.51 mg/dL (17.7 – 1194.3 µmol/L) and urine samples ranging from 0.14 – 141 mg/dL (12.4 – 12434.4 µmol/L). The serum correlation study was performed with 50 unaltered and 5 altered serum samples. The urine correlation study was performed with 42 unaltered and 9 altered urine samples. The correlation analyses are presented below for both serum and urine sample matrices.

Accuracy/Serum samples:
Correlation Coefficient: 0.9981
Slope/Intercept: y = 0.9467x + 0.0643

Accuracy/Urine samples:
Correlation Coefficient: 0.9968
Slope/Intercept: y = 1.0002x – 0.0518

Linearity

The linearity of the procedure is from 0.14-13.56 mg/dL (12 - 1200 µmol/L) in serum and 0.14–141.25 mg/dL (12-12500 µmol/L) in urine. Results below 0.14 mg/dL (12 µmol/L) are invalid. Results that exceed 141.25 mg/dL (12500 µmol/L) should be diluted 10-fold with saline and retested.

Precision

Diazyme Creatinine Liquid Reagents Assay precision was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study, four serum specimens were tested on a Hitachi 917 twice daily, in duplicates over 20 days.

Serum Testing	Within-Run Precision			
	0.75 mg/dL (66.3 µM)	1.41 mg/dL (125 µM)	4.11 mg/dL (346 µM)	10.28 mg/dL (908.7 µM)
No. of Data Points	80	80	80	80
Mean mg/dL (µM)	0.74 (65.4)	1.38 (122.3)	4.04 (357.5)	10.28 (908.7)
SD mg/dL (µM)	0.015 (1.3)	0.015(1.37)	0.029(2.54)	0.015 (1.3)
C_V%	2.1	1.1	0.7	0.1
Serum Testing	**Total Precision**			
	0.75 mg/dL (66.3 µM)	1.41 mg/dL (125 µM)	4.11 mg/dL (346 µM)	10.28 mg/dL (908.7 µM)
No. of Data Points	80	80	80	80
Mean mg/dL (µM)	0.74 (65.4)	1.38 (122.3)	4.04 (357.5)	10.28 (908.7)
SD mg/dL (µM)	0.022(1.9)	0.026(2.29)	0.058(5.11)	0.14(12.4)
C_V%	3.0	1.9	1.4	1.4

Diazyme Creatinine Liquid Reagents Assay precision was also evaluated with urine samples with a modified EP10 protocol. For within-run precision, 21 replicates of commercial urine controls were tested. For total precision, 2 runs of each commercial urine control were performed consecutively for 5 days. The samples were diluted ten-fold with 0.9% saline and tested for Creatinine values. The values were multiplied by the dilution factor (i.e.10) to obtain the final results indicated below.

Urine Testing	Within-Run Precision		
	Level 1	Level 2	Level 3
No. of Data Points	21	21	21
Mean mg/dL (µM)	29.09 (2572)	87.1 (7711)	196.7 (17407)
SD mg/dL (µM)	0.1 (8.84)	0.27 (23.60)	0.90 (79.71)
C_V%	0.36	0.31	0.46
Urine Testing	**Total Precision**		
	Level1	Level 2	Level 3
No. of Data Points	20	20	20
Mean mg/dL (µM)	29.86 (2640)	87.7 (7765)	195 (17265)
SD mg/dL (µM)	0.79 (69.8)	0.67 (59.2)	1.19 (105.2)
C_V%	2.64	0.76	0.60

Interference

Interference for the Diazyme Creatinine Liquid Reagents Assay was evaluated on the Hitachi 917. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglyceride at 1000 mg/dL, Ascorbic Acid at 10 mM, Bilirubin at 40 mg/dL, Bilirubin Conjugate at 30 mg/dL, Hemoglobin at 500 mg/dL. The following substances normally present in urine produced less than 10% deviation at the listed concentrations: Triglycerides at 1000 mg/dL, Ascorbic Acid at 10 mM, Bilirubin at 40 mg/dL, Bilirubin Conjugate at 40 mg/dL, Hemoglobin at 1000 mg/dL.

References

1. Tietz, N. W. (Ed): Fundamentals of Clinical Chemistry, W. B. Saunders Co., Philadelphia, 865 (1982)
2. National Kidney Foundation K/DOQI. Clinical Practice Guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002; 39:S1-S200
3. Badiou S, Dupuy AM, Descomps B, Cristolead, JP. Comparison between the enzymatic vitros assay for creatinine determination and three other methods adapted on the Olympus analyzer, Journal of Clinical Laboratory Analysis 2003:17, 235 - 240
4. Hayes AW. Principles and Methods of Toxicology, Taylor & Francis, 1028 (2001)
5. Cristenson RH, Johnson LJ, Gregory, LC. Appleton and Lange's Outline Review Clinical Chemistry, McGraw-Hill Professional, 118 (2001)
6. Mazzachi BC, Peake MJ, Ehrhardt V. Reference Range and Method Comparison Studies for Enzymatic and Jaffé Creatinine Assays in Plasma and Serum and Early Morning Urine. Clin Lab 2000; 46;53-55

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