

INSTRUCTIONS FOR USE

VITROS CREA Slides

CREA

Creatinine, single-slide

Intended Use

For in vitro diagnostic use only.

VITROS CREA Slides quantitatively measure creatinine (CREA) concentration in serum, plasma, and urine.

Summary and Explanation of the Test

Serum creatinine and urinary creatinine excretion is a function of lean body mass in normal persons and shows little or no response to dietary changes. The serum creatinine concentration is higher in men than in women. Since urinary creatinine is excreted mainly by glomerular filtration, with only small amounts due to tubular secretion, serum creatinine and a 24-hour urine creatinine excretion can be used to estimate the glomerular filtration rate.

Serum creatinine is increased in acute or chronic renal failure, urinary tract obstruction, reduced renal blood flow, shock, dehydration, and rhabdomyolysis. Causes of low serum creatinine concentration include debilitation and decreased muscle mass. Exercise may cause an increased creatinine clearance. The creatinine clearance rate is unreliable if the urine flow is low.

Principles of the Procedure

The VITROS CREA Slide is a dry, multilayered, analytical element coated on a polyester support.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Creatinine diffuses to the reagent layer, where it is hydrolyzed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product.

Following addition of the sample, the slide is incubated at 37°C. During the initial reaction phase, endogenous creatine in the sample is oxidized. Reflectance measurements are then made at 3.85 and 5 minutes. The change in reflectance between the two readings is proportional to the creatinine concentration in the sample.

Test Type	Wavelength	Assay Time and Temperature
Two-point rate	670 nm	Approximately 5 minutes at 37°C

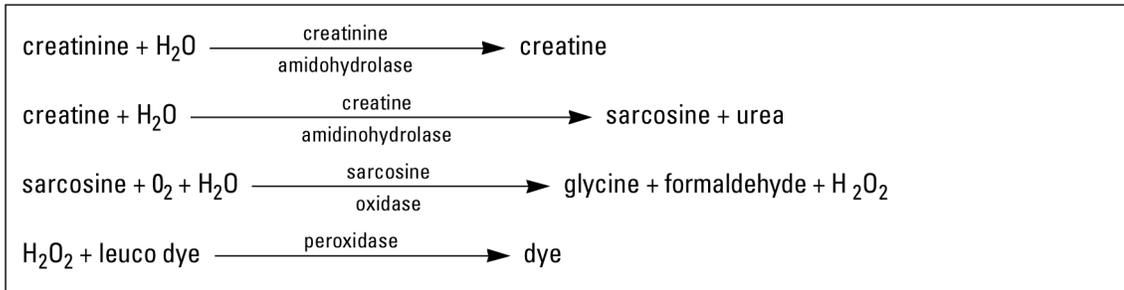
Sample Drop Volume

The volume of the sample drop depends on the format of the slide. For slides with coatings labeled 3201 and above, the sample drop volume is 6 µL. For all other slide formats, the sample drop volume is 10 µL.

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Reaction Sequence



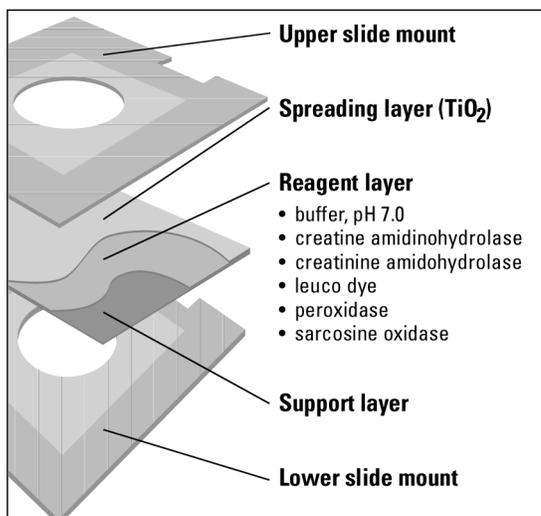
Reagents

Slide Ingredients

Reactive ingredients are creatinine amidohydrolase (*Flavobacterium sp.*, E.C.3.5.2.10); creatine amidohydrolase (*Flavobacterium sp.*, E.C.3.5.3.3); sarcosine oxidase (*Bacillus sp.*, E.C.1.5.3.1); peroxidase (horseradish root, E.C.1.11.1.7); and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole (leuco dye).

Other ingredients include pigment, binders, surfactants, stabilizer, chelator, buffer, dye solubilizer, scavenger, and cross-linking agent.

Slide Diagram



Slide Labeling

The cartridge's outer carton is labeled with the test name, slide lot number, expiration date, and required storage temperature.

Slide Cartridge Handling

CAUTION: Protect the inner wrapper from damage before opening.

- Do not drop a case of cartridges.
- Do not cut into the inner wrapper with a sharp instrument when opening the case.

Slide Storage

Unopened slide cartridges:

Store at or below -18°C (0°F).

NOTE: To reduce cartridge warm-up time or if freezer space is limited, unopened slide cartridges may be stored in the refrigerator at 2°–8°C (36°–46°F) for up to four weeks.

Cartridges in the system's slide supply:

- Leave in the slide supply for no more than two weeks, then replace with a fresh cartridge.
- Leave in the slide supply when the system is turned off for up to two hours.
- Verify performance with control materials:
 - If the system is turned off for more than two hours
 - After reloading cartridges that have been removed from the slide supply and stored for later use

Slide Stability

VITROS CREA Slides are stable until the expiration date on the carton when they are stored and handled as specified.

Slide Preparation

- Remove slide cartridges from storage.
- The slide cartridge must reach room temperature, 18°–28°C (64°–82°F), before it is unwrapped and loaded into the slide supply. Allow the cartridge to warm up at least:
 - 60 minutes after removing from the freezer
 - or
 - 30 minutes after removing from the refrigerator
- Remove the inner wrapper and immediately load into the slide supply.

NOTE: Load the cartridges within 24 hours after they reach room temperature.

Specimen Collection and Preparation

Serum and Plasma Specimens

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Serum; lithium and sodium heparin plasma.

Special Precautions

Do not use specimens obtained through catheters used to infuse hyperalimentation fluid. Refer to "Limitations of the Procedure."

Specimen Collection and Preparation

- Collect specimens using standard laboratory procedures. ^{1,2}
- Refer to the operator's manual section on sample handling for recommended minimum specimen volumes for your system.
- Centrifuge specimens and remove the serum from the clot within 4 hours of collection. ³

Handling and Storage Conditions

- Handle specimens as biohazardous material.
- Handle specimens in stoppered containers to avoid contamination and evaporation.
- Storage requirements: ⁴
 - Store at room temperature up to 5 days
 - Refrigerate up to 30 days
 - Freeze for storage beyond 30 days

Urine Specimens

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Diluted urine.

Specimen Collection and Preparation

- Collect timed specimens using standard laboratory procedures. ⁵
- Keep specimens refrigerated until analysis.
- Refer to the operator's manual section on sample handling for recommended minimum specimen volumes for your system.

Urine Specimen Preparation

Follow this procedure to prepare urine specimens for analysis.

1. Pretreat each specimen by mixing 1 part specimen and 20 parts reagent-grade water.
2. Analyze.
3. Multiply results by 21 to obtain the creatinine concentration.

Handling and Storage Conditions

- Handle specimens as biohazardous material.
- Handle specimens in stoppered containers to avoid contamination and evaporation.
- Storage requirements:⁶
 - Store at room temperature, 18°–28°C (64°–82°F), up to 3 days
 - Refrigerate at 2°–8°C (36°–46°F) up to 5 days
 - Freeze at or below -18°C (0°F) indefinitely

Testing Procedure**Materials Required But Not Provided**

The following items are required to perform the test for CREA:

- VITROS Chemistry Calibrator Kit 1
- Quality-control materials, such as VITROS Performance Verifiers
- For dilution, VITROS 7% BSA

Operating Instructions

Refer to the operator's manual for complete instructions on operation of your system.

Sample Dilution

If samples show creatinine concentrations that exceed the system's reportable (dynamic) range or if they give a DP code (indicating high background density, usually due to an elevated creatine concentration), follow this procedure.

Serum or plasma:

1. Dilute sample with VITROS 7% BSA. An initial twofold dilution is recommended.
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain the original sample's creatinine concentration.

Urine:

1. Make an additional dilution by adding 1 part reagent-grade water to 1 part sample that has been diluted 21-fold.
2. Reanalyze.
3. Multiply the results by 42 to obtain the creatinine concentration in the original sample.

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Calibration

Required Calibrators

VITROS Chemistry Calibrator Kit 1

Calibrator Preparation, Handling, and Storage

Refer to the calibrator package insert for information about reconstitution and use of the Chemistry Calibrator Kit.

Calibration Procedure

Refer to the calibration section of your operator's manual.

When to Calibrate

- Calibrate when the slide lot number changes.
- Calibrate when critical system parts are replaced due to service or maintenance.
- If quality-control results are consistently outside acceptable limits, calibration might be required. Refer to your operator's manual for more detail.
- Calibrate when government regulations require. In the US, CLIA regulations require calibration or calibration verification at least once every six months.

Reference Method

Calibration is traceable to the high performance liquid chromatography method of Ambrose et al.⁷

Calibration Model

Two-point rate (described in your operator's manual).

Quality Control

Procedure Recommendations

- Handle quality-control materials as biohazardous material.
- Analyze quality-control materials in the same manner as patient samples, before or during patient sample processing.
- Analyze control materials at least once per day to verify system performance.
- Choose control levels that check the clinically relevant range.
- Refer to the quality control section in your operator's manual for additional information on quality-control procedures for VITROS Systems.
- Refer to *Internal Quality Control Testing: Principles and Definitions* for general quality-control recommendations.⁸

Quality-Control Material Selection

- VITROS Performance Verifiers are specially formulated for use with VITROS Systems.
- Do not use controls prepared with a diluent containing Tris buffer because results may be lower by approximately 50%.
- Controls that are reconstituted with deionized water should perform acceptably.
- Liquid serum and urine controls often contain high creatine levels and may give DP codes.
- Other control materials may show a difference when compared with other creatinine methods if they:
 - Depart from a true human serum/plasma matrix
 - Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives
- Do not use control materials stabilized with ethylene glycol.

Quality-Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Expected Values and Reporting Results

Reference Interval

	Conventional Units (mg/dL)	SI Units ($\mu\text{mol/L}$)	Alternate Units (mg/L)
Serum			
Females	0.7–1.2	62–106	7–12
Males	0.8–1.5	71–133	8–15
24-hour Urine	800–2800 mg/day*	7000–25000 $\mu\text{mol/day}$ **	—

* Creatinine concentration (mg/dL) x 24-hour volume (dL) = mg/day.

** Creatinine concentration ($\mu\text{mol/L}$) x 24-hour volume (L) = $\mu\text{mol/day}$.

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These reference intervals are the central 95% of results from an internal study of apparently healthy adults from a working population (serum: 90 females, 105 males; urine: 67 subjects). Each laboratory should verify the validity of these intervals for the population it serves.

Reporting Units and Unit Conversion

Conventional Units	SI Units	Alternate Units
mg/dL	$\mu\text{mol/L}$ (mg/dL x 88.4)	mg/L (mg/dL x 10)

Limitations of the Procedure

Known Interfering Substances

Serum/Plasma

- **Creatine:** At a creatinine concentration of 1.5 mg/dL (133 $\mu\text{mol/L}$), creatine greater than 8 mg/dL (707 $\mu\text{mol/L}$) will be flagged with a DP code (because highly elevated creatine concentrations may cause excessive background density). For unflagged samples, residual bias because of creatine will be less than 0.15 mg/dL (13 $\mu\text{mol/L}$). At a creatinine concentration of 14 mg/dL (1237 $\mu\text{mol/L}$), creatine greater than 1 mg/dL (88 $\mu\text{mol/L}$) will be flagged with a DP error code. Residual bias for unflagged samples will be less than 2%.
- **Proline:** Patients receiving hyperalimentation fluids containing proline may show an increase of 0.2 mg/dL (18 $\mu\text{mol/L}$). Do not collect specimens from intravenous fluid lines contaminated with hyperalimentation fluid.
- **Dobutamine:** Specimens contaminated with dobutamine from intravenous fluid have been reported to show a significant negative bias. A dobutamine concentration of 83 $\mu\text{g/mL}$ caused a decrease of 2.7 mg/dL (239 $\mu\text{mol/L}$) from an initial creatinine concentration of 4.8 mg/dL (424 $\mu\text{mol/L}$).⁹
- **Lidocaine:** Patients on long-term lidocaine therapy may show an increase of up to 1.0 mg/dL (88 $\mu\text{mol/L}$) due to a metabolite of lidocaine, N-ethyl glycine (NEG).¹⁰

Table of Known Interfering Substances

The VITROS CREA method was screened for interfering substances. The following substances, when tested at the concentrations indicated, caused the bias shown.

Interferent*	Conventional Units			SI Units		
	Interferent Concentration (mg/dL)	Analyte Concentration (mg/dL)	Average Bias (mg/dL)	Interferent Concentration ($\mu\text{mol/L}$)	Analyte Concentration ($\mu\text{mol/L}$)	Average Bias ($\mu\text{mol/L}$)
Dipyron (Metamizol)	40	1.0	-0.6	1138	88	-53

* It is possible that other interfering substances may be encountered. These results are representative; however, your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

Other Limitations

Some drugs and patient conditions are known to alter creatinine concentrations in vivo. A compilation of this information is available in the literature.^{11, 12}

Performance Characteristics

Reportable Range (Dynamic Range)

	Conventional Units (mg/dL)	SI Units (μmol/L)	Alternate Units (mg/L)
Serum	0.05–14.00	4–1238	0.5–140
Urine	1.05–346.5*	84–30639*	10.5–3465*

*After multiplying by a dilution factor of 21.

Refer to Sample Dilution under “Testing Procedure” for out-of-range samples.

Sensitivity

The lower limit of the reportable (dynamic) range is 0.05 mg/dL (4 μmol/L) for serum and 1.05 mg/dL (84 μmol/L) for urine.

Precision

Precision was evaluated with quality-control materials on VITROS 250, 700, and 950 Chemistry Systems following NCCLS Protocol EP5.¹³

These results are guidelines. Variables such as instrument maintenance, environment, slide handling/storage, control material reconstitution, and sample handling can affect the reproducibility of test results.

CREA Precision (Serum)

SYSTEM	Conventional Units (mg/dL)			SI Units (μmol/L)			Within Lab CV%**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**			
VITROS 250	1.0	0.01	0.01	87	0.9	0.9	1.3	79	20
	5.9	0.05	0.11	523	4.4	9.7	1.8	78	20
VITROS 700	0.2	0.00	0.01	18	0.0	0.9	5.4	92	23
	0.9	0.01	0.01	80	0.9	0.9	1.2	90	23
	5.6	0.04	0.05	495	3.5	4.4	1.0	91	23
VITROS 950	0.9	0.01	0.01	81	0.7	0.9	1.1	90	23
	5.6	0.05	0.06	499	4.1	5.7	1.1	92	23

* Within Day precision was determined using two runs/day with two to three replications.

** Within Lab precision was determined using a single lot of slides and calibrating weekly.

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CREA Precision (Urine)

SYSTEM	Conventional Units (mg/dL)			SI Units (μmol/L)			Within Lab CV%**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**			
VITROS 250	69.7	1.11	1.91	6160	97.8	168.8	2.7	80	20
	91.4	1.53	2.39	8083	135.0	211.1	2.6	88	22
	140.0	2.91	5.56	12373	257.3	491.9	4.0	84	21
	213.0	2.47	4.51	18827	218.5	398.6	2.1	88	22
VITROS 700	87.5	0.74	2.40	7735	65.4	212.2	2.7	90	23
	229.4	1.35	6.04	20279	119.3	533.9	2.6	88	23
VITROS 950	87.6	0.79	2.54	7744	69.8	224.5	2.9	90	23
	230.3	1.39	5.62	20359	122.9	496.8	2.4	88	23

* Within Day precision was determined using two runs/day with two to three replications.

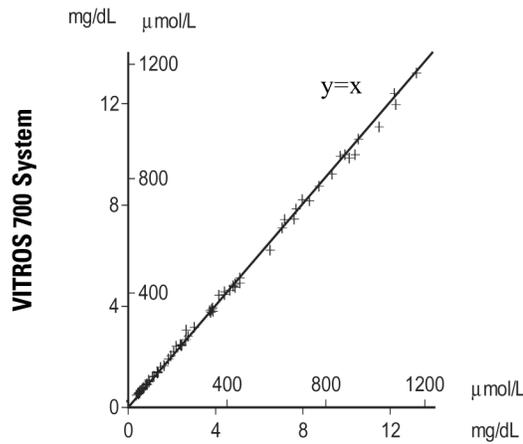
** Within Lab precision was determined using a single lot of slides and calibrating weekly.

Accuracy

The plots and tables show the results of a comparison of serum and urine specimens analyzed on the VITROS 700 System with those analyzed using the high performance liquid chromatography reference method.⁷

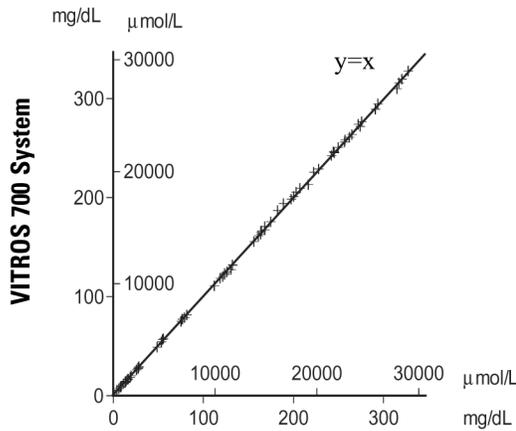
The tables also show the results of comparisons of the VITROS 250 and 950 Systems with the VITROS 700 System.

CREA/Serum



High Performance Liquid Chromatography Reference Method⁷

CREA/Urine



High Performance Liquid Chromatography Reference Method⁷

Method Comparison (Serum)

	n	Slope	Correlation Coefficient	Conventional Units (mg/dL)			SI Units (μmol/L)		
				Range of Sample Concentration	Intercept	Sy.x	Range of Sample Concentration	Intercept	Sy.x
700 System vs. reference method	68	0.98	0.999	0.4–13.2	0.09	0.13	35–1167	7.96	11.49
250 System vs. 700 System	52	1.03	0.999	0.9–13.3	-0.08	0.13	80–1176	-7.07	11.49
950 System vs. 700 System	115	1.00	0.999	0.5–13.2	-0.01	0.03	44–1167	-0.88	2.65

Method Comparison (Urine)

	n	Slope	Correlation Coefficient	Conventional Units (mg/dL)			SI Units (μmol/L)		
				Range of Sample Concentration	Intercept	Sy.x	Range of Sample Concentration	Intercept	Sy.x
700 System vs. reference method	74	1.00	0.999	4.6–327.6	0.27	1.54	407–28960	23.82	135.95
250 System vs. 700 System	59	1.04	0.999	30.7–314.4	-4.97	3.05	2714–27793	-439.35	269.62
950 System vs. 700 System	109	1.02	0.999	9.7–337.5	-0.81	1.27	857–29835	-71.60	112.27

Specificity

The following substances were tested with VITROS CREA Slides and found not to interfere (bias < 0.1 mg/dL, < 8.8 μmol/L):

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Compound	Concentration
Acetoacetate	30 mmol/L
Ampicillin	1.5 mg/dL
Amikacin	1.5 mg/dL
Ammonium Chloride	1 mmol/L
Amphotericin B	1.5 mg/dL
Ascorbic Acid	3 mg/dL
Bacitracin	1.5 mg/dL
Bicarbonate	40 mmol/L
Bilirubin	20 mg/dL
Bleomycin Sulfate	1.5 mg/dL
Carbenicillin	1.5 mg/dL
Cefazolin	1.5 mg/dL
Cephalothin	1.5 mg/dL
Cephaloridine	1.5 mg/dL
Cephaloglycin	1.5 mg/dL
Cephalexin	1.5 mg/dL
Cephardine	1.5 mg/dL
Cleocin	1.5 mg/dL
Cloxacillin	1.5 mg/dL
Demeclocycline	1.5 mg/dL
Dextran	1000 mg/dL
Dicloxacillin	1.5 mg/dL
Doxycycline	1.5 mg/dL
Di-cycloserine	1.5 mg/dL
Dilantin	2 mg/dL
Ethambutol	1.5 mg/dL
Ethanol	300 mg/dL
Furazolidone	1.5 mg/dL
5-Fluorocytosine	5 mg/dL
Gentamicin	1.5 mg/dL
Glucose	600 mg/dL
Glutathione	1 mg/dL
Hemoglobin	150 mg/dL
Hypaque	500 mg/dL
Kanamycin	1.5 mg/dL

Compound	Concentration
Isoniazid	1.5 mg/dL
Limcomycin	1.5 mg/dL
Methicillin	1.5 mg/dL
6-Mercaptopurine	1.5 mg/dL
Minocycline	1.5 mg/dL
Nalidixic Acid	1.5 mg/dL
Nafcillin	1.5 mg/dL
Neomycin	1.5 mg/dL
Nitrofurantoin	1.5 mg/dL
Oxacillin	1.5 mg/dL
Oxytetracycline	1.5 mg/dL
Penicillin-g	1.5 mg/dL
Phenobarbital	3 mg/dL
Phenoxymethylpenicillanic acid	1.5 mg/dL
pH	6.8/8.8
Polymyxin B sulfate	1.5 mg/dL
Polymyxin E	1.5 mg/dL
Potassium	8 mEq/L
Rifampicin	1.5 mg/dL
Spectinomycin	1.5 mg/dL
Streptomycin sulfate	1.5 mg/dL
Sulfachloropyridazine	1.5 mg/dL
Sulfamethoxypyridazine	1.5 mg/dL
Sulfamethoxazole	1.5 mg/dL
Sulfisoxazole	1.5 mg/dL
Sulfadiazine	1.5 mg/dL
Sulfathiazole	6 mg/dL
Tetracycline	1.5 mg/dL
Ticarcillin	1.5 mg/dL
Tolbutamide	22 mg/dL
Triglycerides	800 mg/dL
Vancomycin	1.5 mg/dL
Urea Nitrogen	100 mg/dL
Uric Acid	15 mg/dL

Urine

The following preservatives have been tested and demonstrated an effect of less than 2% on creatinine results:

- Thymol
- Toluene
- Boric acid
- Glacial acetic acid
- 12N HCl
- NH₄OH
- Bromide
- Iodide
- 5% NaOH

References

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Revision History

Date of Revision:	Version:	Description:
2002APR19	1.0	New format, technically equivalent to 2001OCT18

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature _____
Obsolete Date

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