COD 11795 2 x 25 mL	COD 11895 1 x 25 mL
STORE AT 2-8ºC	
Reagents for measurement of citrate concentration Only for *in vitro* use in the clinical laboratory	

CITRATE

Automated Systems

BioSystems REAGENTS & INSTRUMENTS

CE

CITRATE
CITRATE LYASE/MALATE DEHYDROGENASE

PRINCIPLE OF THE METHOD

Citrate in the sample consumes, by means of the coupled reactions described below, NADH that can be measured by spectrophotometry[1].

$$\text{Citrate} \xrightarrow{\text{CL}} \text{Oxaloacetate} + \text{Acetate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{malate} + \text{NAD}^+$$

CONTENTS

	COD 11795	COD 11895
A. Reagent	2 x 20 mL	1 x 20 mL
B1. Reagent	2 x 5 mL	1 x 5 mL
B2. Reagent	2 x 5 mL	1 x 5 mL
S. Standard	1 x 3 mL	1 x 3 mL

COMPOSITION

A. Reagent. Tris 40 mmol/L, NADH 0.4 mmol/L, preservatives, pH 9.5.

B1. Reagent. PIPES 600 mmol/L, preservatives, pH 6.5.
WARNING: H317: May cause an allergic skin reaction. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

B2. Reagent. Malate dehydrogenase > 40 KU/L, citrate lyase > 1 KU/L, after reconstitution.

S. Citrate standard. Citric acid 100 mg/dL (5.20 mmol/L). Aqueous primary standard.
WARNING: H317: May cause an allergic skin reaction. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank lower than 1.2 at 340 nm (1 cm cuvette).
– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent A is ready to use

Reagent B: Reconstitute the Reagent B2 with the contents of the Reagent B1 vial. Mix gently. Stable for 1 month at 2-8ºC.

Reagents open and kept in the refrigerated compartment of the analyzer are stable 3 weeks.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC.
– Analyzer, spectrophotometer or photometer able to read at 340 ± 20 nm.

SAMPLES

Seminal plasma: Allow semen to liquefy at 37ºC for 30 minutes. Centrifuge to separate the spermatozoa[2]. Citrate is stable in the seminal plasma for 6 months at -20°C.
Urine: Collect a 24-hour urine specimen using HCl as a preservative. Stable for 30 days at 2-8ºC. Centrifuge or filter before testing.

PROCEDURE

Sample dilution (seminal plasma)

The standard and urine samples do not require pre-treatment.

1. Pipette into a test tube:

Seminal plasma	200 µL
Distilled water	800 µL

2. Shake thoroughly. The diluted sample is stable 8 hours at 15-25ºC, 24 hours at 2-8ºC.

Manual procedure

1. Bring the reagents to room temperature.
2. Pipette into labelled test tubes:

	Blank	Standard	Sample
Distilled water	20 µL	—	—
Citrate Standard (S)	—	20 µL	—
Sample	—	—	20 µL
Reagent A	1.2 mL	1.2 mL	1.2 mL
Reagent B	300 µL	300 µL	300 µL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard, the Sample and the Blank at 340 nm. The colour is stable for at least 30 minutes.
5. Calculate the citrate concentration using the following formula:

$$\frac{A_{Sample} - A_{Blank}}{A_{Standard} - A_{Blank}}$$

Seminal plasma	Urine
x 500 = mg/dL citrate x 26,1 = mmol/L citrate	x 100 = mg/dL citrate x 5.21 = mmol/L citrate

Automated procedure (Note 1)

		A25	A15
GENERAL	Test name	CITRATE	CITRATE
	Analysis mode	Differential bir	Differential bir
	Sample type	SEM/URI	SEM/URI
	Units	mg/dL	mg/dL
	Reaction type	decreasing	decreasing
	Decimals	0	0
	No. of replicates	1	1
	Test name in patient report	-	-
PROCEDURE	Reading	Monochromatic	Monochromatic
Volumes	Sample	4	4
	Reagent 1	240	240
	Reagent 2	60	60
	Washing	1.2	1.2
	Predilution factor	-	-
	Postdilution factor	2	2
Filters	Main	340	340
	Reference	-	-
Times	Reading 1	75 s	72 s
	Reading 2	390 s	384 s
	Reagent 2	90 s	96 s
CALIBRATION	Calibration type	specific	specific
	Calibrator replicates	3	3
	Blank replicates	3	3
	Calibration curve	-	-
OPTIONS	Blank absorbance limit	1.200	1.200
	Kinetic blank limit	-	-
	Linearity limit	1250 (urine: 250)	1250 (urine: 250)

It is recommended to do a reagent blank every day and a calibration at least every 6 weeks, after reagent lot change or as required by quality control procedures.

REFERENCE VALUES

Seminal plasma: > 300 mg/dL = > 15.6 mmol/L[3]; > 10 mg/ejaculate = > 52 µmol/ejaculate[4]

Urine[3]: Male: 116-924 mg/24h = 0.60-4.81 mmol/24-h
Female: 250-1160 mg/24-h = 1.30-6.04 mmol/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Fertility Biochemistry Control (Cod. 18053) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

The following data were obtained using an A25 analyser. Results are similar with A15. Details on evaluation data are available on request.

– Linearity limit: 1250 mg/dL = 65 mmol/L (seminal plasma); 250 mg/dL = 13 mmol/L (urine). For higher values dilute pretreated sample 1/2 with distilled water and repeat measurement.
– Detection limit: 19 mg/dL = 1.0 mmol/L (seminal plasma); 3.8 mg/dL = 0.2 mmol/L (urine).
– Repeatability (within run):

Mean Concentration (seminal plasma)	Mean Concentration (urine)	CV	n
200 mg/dL = 10.4 mmol/L	40 mg/dL = 2.08 mmol/L	2.5 %	20
750 mg/dL = 39.0 mmol/L	150 mg/dL = 7.80 mmol/L	1.1 %	20

– Reproducibility (run to run):

Mean Concentration (seminal plasma)	Mean Concentration (urine)	CV	n
200 mg/dL = 10.4 mmol/L	40 mg/dL = 2.08 mmol/L	3.8 %	25
750 mg/dL = 39.0 mmol/L	150 mg/dL = 7.80 mmol/L	3.3 %	25

– Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.

DIAGNOSTIC CHARACTERISTICS

Citrate is produced by the prostate gland and is found in seminal plasma. Citrate is the main anion in the prostate gland and plays an important role as an ion chelating agent. The measurement of its concentration in seminal plasma is used as a tracer in determining the secretory function of the prostate gland. Low values indicate an abnormal disruption of the secretory function of the gland, possibly as a result of obstruction of the ducts due to inflammation of acute or chronic nature[3,4].

Urinary citrate inhibits stone formation by forming soluble complexes with calcium. Excretion is reduced in the calcium stone-forming population. Urinary citrate measurement may be of value in the assessment of stone-forming risk[3].

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
4. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge university press, 3th ed, 1992.