BIOLABO
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UREA Colorimetric Method

Reagent for quantitative determination of urea in human serum and plasma or urines.

REF 80221	**R1** 1 x 125 mL	**R2** 1 x 1.25 mL	**R3** 1 x 31 mL	**R4** 1 x 10 mL
REF 80321	**R1** 1 x 500 mL	**R2** 1 x 5 mL	**R3** 1 x 125 mL	**R4** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (5)

More than 90% of urea is excreted through the kidneys in urines. Measurement of the plasma or serum urea concentration is widely regarded as a test of renal function. However, a number of nonrenal factors also influence the circulating urea concentration: Urea increased level occurs when proteins catabolism is accelerated, burns, stress, myocardial infarction... Urea is decreased in acute liver destruction and is accompanied with increased ammonium level. Urea level is generally studied in conjunction with creatinine level (urea/creatinine ratio) to refine post-renal or pre-renal diagnosis.

PRINCIPLE (4)

Enzymatic and colorimetric method based on the specific action of urease which hydrolyses urea in ammonium ions and carbon dioxide. Ammonium ions then form with chloride and salicylate a blue-green complex. This coloration, proportional to urea concentration in the specimen, is measured at 600 nm.

REAGENTS COMPOSITION

Vial R1 **SALICYLATE**

Salicylate	31	mmol/L
Nitroprussiate	1.67	mmol/L

Vial R2 **UREASE**

Urease	≥ 15	KUI/L

Vial R3 **BASE**

Sodium hypochlorite	7	mmol/L
Sodium hydroxide	62	mmol/L

Before dilution: Xi, R36/38, Irritating to eyes and skin
S24-25-26-28: Avoid contact with skin and eyes. After contact with skin, rinse immediatly with plenty of water. After contact with eyes, rinse immediatly with plenty of water and seek medical advice
Once diluted: None

Vial R4 **STANDARD** Urea 40 mg/dL (6.66 mmol/L)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Add contents of vial R2 (Urease) into vial R1 (Salicylate). Mix gently by inversion.

Alkaline (vial R3):

Procedure n°1 and n°2 (manual): Dilute (1 + 3) with demineralised water

Procedure n°3 (manual or automatic): Ready to use

Standard (vial R4): transfer the requested quantity, recap and store at 2-8°C.

STABILITY AND STORAGE

Store at 2-8°C, well recap in the original vial and away from light.

- Unopened, reagents are stable until expiry date stated on the label when stored and used as described in the insert.
- Once reconstituted, working reagent (R1+R2) is stable for 1 month when free from contamination.
- Once opened, Base (vial R3) diluted ¼ is stable for 3 months when free from contamination.
- Once opened, the contents of vial R4 is stable for at least 3 months when free from contamination.

Discard any reagent if cloudy or if absorbance of blank against water at 600 nm > 0.100.

Don't use working reagent or diluted contents of vial R3 after expiry date stated on the label of the kit.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum or heparinised plasma. Avoid fluoride or ammonium as anticoagulant which interfere with the assay.

Urea is stable in serum or plasma for:

- 24 h at room temperature.
- several days at 2-8°C.
- at least 2-3 months frozen.

24h Urine: diluted (1+19) with demineralised water before assay.

Urea is stable in urines for:

- 4 days at 2-8°C.

Add antibacterial agent as Thymol to improve the stability.

INTERFERENCES (3)

No interference of assayed substances (ascorbic acid, bilirubin, haemoglobin, triglycerides) with the test.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with

CALIBRATION

- Standard enclosed in the kit (vial R4) or BIOLABO Multicalibrator REF 95015 traceable to SRM 909b.
- Or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

It is recommended to calibrate in the following cases :

1. When changing batch of reagent.
2. After maintenance operations on the instrument.
3. If control values are out of range, even after using a new vial of fresh control serum.

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Other assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

In serum and plasma	mg/dL	[mmol/L]
In cord	45-86	[7.5-14.3]
Premature	6-54	[1.1-8.9]
< 1 year	9-41	[1.4-6.8]
Children	11-39	[1.8-6.4]
18-60 years	13-43	[2.1-7.1]
60-90 years	17-49	[2.9-8.2]
> 90 years	21-66	[3.6-11.1]
In urines	26-43 g/24 h	[0.43-0.71 mol/24 h]

To calculate blood urea nitrogen (BUN): multiply the value of urea (mg/dL) by 0.467.

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE CHARACTERISTICS (Procedure n°1)

Within run N = 20	*Normal level*	*High level*
Mean mg/dL	40	141.6
S.D. mg/dL	0.76	1.66
C.V. %	1.89	1.17

Between run N = 20	*Normal level*	*High level*
Mean mg/dL	35	111
S.D. mg/dL	1.58	3.44
C.V. %	4.5	3.1

Detection limit: approximately 10 mg/dL.

Comparison with commercially available reagent: y = 0.9816 + 0.87

r = 0.9961

	Sensitivity for 100 mg/dL at 600 nm
Procedure n°1	Approx. 0.400 abs
Procedure n°2	Approx. 0.800 abs
Procedure n°3	Approx. 0.700 abs

Note: Sensitivity is higher at upper wavelength and lower at inferior wavelength.

LINEARITY

Procedure n°1 and n°3:
The reaction is linear up to 250 mg/dL (41.7 mmol/L).
Procedure n°2: The reaction is linear up to 125 mg/dL (20.9 mmol/L).
Above, dilute the specimen with saline solution and reassay taking into account dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Procedure n°1

Pipette into test tubes	Blank	Standard	Assay
Working reagent (R1+R2)	1 mL	1 mL	1 mL
Demineralised water	5 µL		
Standard		5 µL	
Specimen (Note 1)			5 µL
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C			
Base (vial R3) diluted ¼	1 mL	1 mL	1 mL
Mix. Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm (590-610) against blank (Note 3). Reaction coloration is stable for 2 hours.			

Procedure n°2

Pipette into test tubes	Blank	Standard	Assay
Working reagent (R1+R2)	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen (Note 1)			10 µL
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C			
Base (vial R3) diluted ¼	1 mL	1 mL	1 mL
Mix. Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm (590-610) against blank (Note 3). Reaction coloration is stable for 2 hours.			

Procédure n°3 (alcalin PUR)

Pipette into test tubes	Blanc	Etalon	Dosage
Working reagent (R1+R2)	1 mL	1 mL	1 mL
Demineralised water	5 µL		
Standard		5 µL	
Specimen (Note 1)			5 µL
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C			
Base (vial R3) PUR	250 µL	250 µL	250 µL
Mix. Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm (590-610) against blank (Note 3). Reaction coloration is stable for 2 hours.			

1. Serum, plasma or urines diluted (1+19) with demineralised water.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
3. The test may be performed **at 578 nm**. In this case, Procedure n°2 is linear up to 300 mg/dL.

CALCULATION

Calculate the result as follows:

Serum and plasma:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Urines diluted (1+19):

Multiply the result by 20 (dilution factor).

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1239-1241.*

(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 1096-1099.*

(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1990) p. 3-599 to 3-609*

(4) *SEARCY R.L., REARDON J.E., FOREMAN J.A., Amer. J. Méd. Techn. 1967, 33, 15-20.*

(5) *Bernard S. Bioch. clin. Diagnostics médicaux chirurgicaux 2ème éd. p.143-144. Ed. Maloine PARIS (1989).*

(4) *SRM: Standard Reference Material ®*