**BIOLABO**
**www.biolabo.fr**
**MANUFACTURER:**
**BIOLABO SAS,**
Les Hautes Rives
02160, Maizy, France

# **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

**TECHNICAL SUPPORT AND ORDERS**
**Tel: (33) 03 23 25 15 50**
**support@biolabo.fr**
**Latest revision: www.biolabo.fr**

CE

IVD

Made In France

I: corresponds to significant modifications

## I INTENDED USE

This reagent is designated for professional use in laboratory (semi-automated or automated method).
It allows quantitative determination of of urea in human serum and plasma or urines to screen its level.

## GENERALITIES (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

**R1** **UREA** Salicylate

| | | |
|---|---|---|
| Salicylate | 31 | mmol/L |
| Nitroprussiate | 1.67 | mmol/L |

Warning: Eye Irrit. 2: H319 – causes serious eye irritation

P280: wear protective gloves/eye protection/face protection
P305+P351+P338: IF IN EYES, Rinse cautiously with water several minutes; Remove contact lenses, if present and easy to do. Continue rinsing.
Classification due to sodium salicylate 1 - <2.5%. For more details, refer to safety data sheet (SDS).

**R2** **UREA** Urease

| | | |
|---|---|---|
| Urease | ≥ 15 | KUI/L |

Working reagent (R1+R2) is not classified as dangerous.

**R3** **UREA** Base

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

Before dilution: Danger: Met. Corr. 1: H290 – may be corrosive to metals Skin CORR. 1B ; H314 – causes severe skin burnsand eye damage.
P280: wear protective gloves/eye protection/face protection
P305+P351+P338: IF IN EYES, Rinse cautiously with water several minutes; Remove contact lenses, if present and easy to do. Continue rinsing.
Classification due to sodium hydroxide, sodium hypochlorite 1 - <2.5%. For more details, refer to safety data sheet (SDS).
Once diluted, reagent is not classified as dangerous according to regulation 1272/2008/EC.

**R4** **UREA** Standard Urea 40 mg/dL (6.66 mmol/L)

Reagents R2 and R4 are not classified as dangerous according to regulation 1272/2008/EC.

## SAFETY CAUTION

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

## REAGENTS PREPARATION

Working reagent: Add contents of vial R2 into vial R1 (Salicylate). Mix gently by inversion.

Base (vial R3): Dilute (1 + 3) with demineralised water
In some cases (automated procedure), may be used pur

Standard (vial R4): ready for use.

## STABILITY AND STORAGE

**Stored away from light, well caped in the original vial at 2-8°C, and used as described, reagents are stable:**

Unopened:

- Until expiry date stated on the label of the kit

Once opened, without contamination:

- Working reagent (R1 + R2) is stable 1 month.
- Base (vial R3) dilutd ¼ is stable 1 month.
- Standard (vial R4): Transfer the requested quantity, recap, and store at 2-8°C

- Discard any reagent if cloudy or if if blank at 600 nm > 0.100.

Don't use reagents after expiry date.

## 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.

## LIMITS (3)

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

## I MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

## CALIBRATION (4)

- REF 95015 Multicalibrator traceable to SRM 909c.
- With manual procedure only: Standard (vial R4)

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

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

## QUALITY CONTROL

- REF 95010: Exatrol N Level 1
- REF 95011: Exatrol P Level 2
- 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. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and re-assay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

## REFERENCE INTERVAL (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] |

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

## PERFORMANCES

On Cobas Mira, 37°C, 600 nm

Repeatability:

| *Within run N = 20* | *Level1* | *Level2* | *Level3* |
|---|---|---|---|
| Mean mg/dL | 25 | 59 | 141 |
| S.D. mg/dL | 0,5 | 0,78 | 1,27 |
| C.V. % | 2.0% | 1.3% | 0.9% |

Reproducibility:

| *Between run N = 20* | *Level1* | *Level2* | *Level3* |
|---|---|---|---|
| Mean mg/dL | 14 | 47 | 153 |
| S.D. mg/dL | 0,77 | 1,55 | 3,52 |
| C.V. % | 5.5% | 3.3% | 2.3% |

Manual procedure:

Measuring range: up to 250 mg/dL (41.7 mmol/L)

Detection limit: approximately 10 mg/dL.

Analytical sensitivity (1 cm): approx. 0.400 abs. (100 mg/dL)

Comparison with commercially available reagent:

y = 0.9816 + 0.87 r = 0.9961

Interferences:

| | |
|---|---|
| Total bilirubin | No interference up to 583µmol/L |
| Ascorbic acid | No interference up to 2500 mg/dL |
| Glucose | No interference up to 1110 mg/dL |
| Turbidity | No interference of the turbidity up to 0.333 abs. |
| Haemoglobin | No interference up to 248 µmol/L |

Other substances may interfere (see § Limits)

## PROCEDURE

Manual procedure

Let stand reagents and specimens at room temperature.

| 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. | | | |

1- Performances with manual procedure should be validated by user.
2- For better sensitivity, specimen volume may be enhanced to 10 µL, with lowest linearity at 600 nm
3- Sensitivity is higher at upper wavelength and lower at inferior wavelength
4- On Kenza Max at 578 nm, specimen volume is 10 µL to optimize the couple sensitivity/linearity
5- Above linearity limit dilute specimen with saline solution and re-assay considering dilution factor.
6- Specific applications for automatic analyzers are available on request.

## CALCULATION

Manual procedure:

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).

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

Automatic Biochemistry analyzer:

The analyzer provides directly calculated result.

For more details about calibration and calculation of results, refer to User's manual and specific application

## REFERENCES


(1) *TIETZ N.W. Textbook 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* ®


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|---|---|---|---|---|---|
| Manufacturer | Expiry date | In vitro diagnostic | Storage temperature | Dematerialized water | Biological risk |
| Product Reference | See Insert | Batch number | Store away from light | Sufficient for | Dilute with |