

# UREA

## IFCC UV

### Intended use:

Enzymatic in vitro test for the quantitative determination of urea in human serum, plasma and urine.

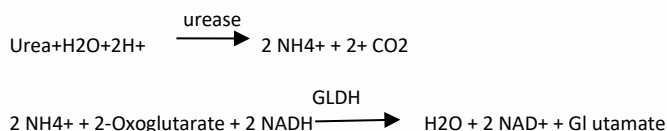
### Summary:

The determination of urea is the most widely used test for the evaluation of kidney function. The test is frequently used in conjunction with the determination of creatinine for the differential diagnosis of prerenal hyperuremia (cardiac decompensation, water depletion increased protein catabolism), renal hyperuremia (glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, tubular necrosis) and postrenal hyperuremia (obstructions of the urinary tract).

Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body. In 1914 Marshall introduced an assay based on the enzyme urease for determining urea in blood. The ammonia released from urea by urease was measured titrimetrically. Numerous other techniques have since been employed to measure the ammonia produced. These include Bertholot's indophenol assay and the reaction of ammonia with Nessler's reagent. Subsequent modifications have been published by Fawcett and Scott and by Chaney and Marbach. In 1995, Talke and Schubert published a totally enzymatic procedure for the determination of urea using the coupled urease/glutamate dehydrogenase (GLDH) enzyme system. The Analyticon UREA/BUN assay is based on the completely enzymatic method. It has been optimized for automatic analyzers that permit kinetic measurements.

### Test principle:

Urea is hydrolysed in presence of urease to produce ammonia and CO<sub>2</sub>. The ammonia produced combines with 2-oxoglutarate and NADH in presence of GLDH to yield glutamate and NAD.



The decrease in absorbance due to consumption of NADH is measured kinetically.

### Reagent concentration:

R1:  
BICIN\* buffer pH 7.6 50 mmol/l  
GLDH ≥ 0.80 U/l  
Urease ≥ 12 U/ml

R2:  
TRIS\*\* buffer pH 9.6 100 mmol/l  
2-oxoglutarate 8.3 mmol/l  
NADH ≥ 0.23 mmol/l

\* BICIN = N,N-bis(2-hydroxyethyl)-glycine  
\*\* Tris(hydroxymethyl)aminomethane

### Preparation and stability:

R1: ready for use  
R2: ready for use  
The reagents are stable up to the expiry date on the label when stored at +2°C to +8°C.

Onboard stability: R1 28 days R2 28 day

### Specimen:

**Serum**  
Collect serum using standard sampling tubes.  
Li-heparin, Na-heparin or K-EDTA plasma. Do not use ammonium heparin.  
Stability: 7 days at +20°C to +25°C  
7 days at +2°C to +8°C  
1 year at -20°C

**Urine**  
Collect urine without using preservatives.  
Stability: 2 days at +20°C to +25°C  
7 days at +2°C to +8°C  
1 month at -20°C

- Urine samples are automatically diluted 1 + 19 with 0.9% NaCl or distilled water in the analyzer. The respective dilutions are taken into account in the calculation of results.
  - Analyzers without automatically sample dilution Manually dilute urine samples with 0.9 % NaCl or distilled water (e.g. 1 + 10). Multiply the result by the appropriate dilution factor (e.g. 11).
- Centrifuge samples containing precipitate before performing the assay.

### Limitations - interference:

- Criterion: Recovery within 10% of initial value.
- Icterus: No significant interference up to an I index of 100 (approximate conjugated and unconjugated bilirubin concentration 100 mg/dl)
- Hemolysis: No significant interference up to an H Index of 800 (approximate hemoglobin concentration: 800 mg/dl).
- Lipemia (Intralipid): No significant interference up to an L index of 1200 (approximate triglycerides concentration: 2400 mg/dl).
- There is poor correlation between turbidity and triglycerides concentration
- Ammonia produced on the cuvette during a GLDH or lactate UV determination interferes with the UREA/BUN assay. The urea/BUN must therefore not be installed on the analyzers together with reagents for the GLDH or lactate UV test. In urine endogenous ammonium ions interfere with the urea/BUN assay. Elevated concentrations can occur under acidic conditions (e.g. acidosis.)
- Great care must be taken to prevent ammonia contamination of the specimens and calibrators to be analyzed for urea/urea nitrogen.

### Testing procedure:

Applications for automated systems are available on request.  
Materials provided

- Working solutions as described above
- Additional materials required
- Calibrators and controls as indicated below
  - 0.9% NaCl

| Manual procedure for serum start:                                                                               |                   |
|-----------------------------------------------------------------------------------------------------------------|-------------------|
| Wavelength:                                                                                                     | 340nm             |
| Temperature:                                                                                                    | +25 / +30 / +37°C |
| Cuvette:                                                                                                        | 1 cm light path   |
| Zero adjustment:                                                                                                | against water     |
| R1                                                                                                              | 800 uL            |
| Serum/Plasma                                                                                                    | 10 uL             |
| Mix, incubate 1-5 min. Then Add;                                                                                |                   |
| R2                                                                                                              | 200 uL            |
| Mix, incubate for 40 seconds and read A1.<br>Perform other 4 readings at 20 sec. intervals.<br>calculate A/min. |                   |

### Calculation:

Conversion into SI-units, relation between urea and urea-nitrogen:  
mg/dl x 0,166 = mmol/l (urea)  
mg/dl urea x 0,467 = mg/dl urea-nitrogen



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### Measuring/reportable range

Serum/plasma : 5 - 300 mg/dl (0.83 to 66.4 mmol/l) urea or 2 – 186 mg/dl urea nitrogen. Determine samples with higher concentrations via the rerun function. On instruments without rerun function, manually dilute samples with 0.9% NaCl or distilled water (e.g. 1+2). Multiply the result by the appropriate dilution factor (e.g. 3).

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### Expected values:

Serum/plasma

10 – 50 mg/dl

Reference ranges for children are given in the brochure "Reference ranges for adults and children; preanalytical considerations" by Heil W. Koberstein R, Zawta B. (published by Boehringer Mannheim GmbH 1997).

Morning urine

847 – 2967 mg/dl (141 – 494 mmol/l)

24-hour urine

10 – 35 g/24h (170 – 580 mmol/24h), corresponding to

670 – 2300 mg/dl (110 – 390 mmol/l)

The expected values are influenced by daily take-up of proteins on relation to the body weight.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

For diagnostic purposes the urea results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

### Analytical sensitivity (lower detection limit)

Detection limit: 5 mg/dl (0.83 mmol/l)

The lower detection limit represents the lowest measurable urea activity that can be distinguished from zero.

### Precision:

Reproducibility within run was determined using human samples and controls (n = 20). The following results were obtained:

| Serum       |            |          |      |
|-------------|------------|----------|------|
| Within run  |            |          |      |
| Sample      | Mean mg/dl | SD mg/dl | CV % |
| Sample1     | 41.8       | 1.34     | 3.21 |
| Sample2     | 98.4       | 1.63     | 1.66 |
| Sample3     | 142        | 3.03     | 2.13 |
| Serum       |            |          |      |
| Between day |            |          |      |
| Sample      | Mean mg/dl | SD mg/dl | CV % |
| Sample1     | 43.5       | 1.34     | 3.07 |
| Sample2     | 65.6       | 2.05     | 3.12 |
| Sample3     | 140.2      | 3.26     | 2.32 |

### Method comparison:

A comparison of the BIOANALYTIC UREA/BUN (y) with a commercial obtainable assay (x) gave the following result:

$$y = 0,993 x + 0,389;$$

$$r = 0,998$$

### Quality Control:

Control Serum:

BIOCON N 5 x 5 ml #B10814

BIOCON P 5 x 5 ml #B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

### Calibration:

S1: 0.9% NaCl

S2: BIOCAL H 5 x 3 ml #B11895

### Literature:

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- Tietz NW. Clinical Guide to Laboratory Tests 3rd ed. Philadelphia, Pa. WB Saunders Company. 1995:624, 622 - 629
- Tietz NW. Fundamentals of Clinical Chemistry Philadelphia, Pa: WB Saunders Co 1976:991

### Order information (Cat No.) :

|         |         |        |        |        |        |
|---------|---------|--------|--------|--------|--------|
| CC490   | OL490   | B24290 | B28291 | B33291 | B80290 |
| KL490   | CR490   | B25290 | B30290 | B34290 | B80291 |
| BURE500 | BURE125 | B25291 | B30291 | B35290 | B80292 |
| AB490   | B21290  | B27290 | B31290 | B36290 |        |
| SH490   | B21291  | B27291 | B32290 | B37290 |        |
| BURE250 | B22290  | B28290 | B33290 | B42290 |        |

### Manufacturer





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## SYMBOLS

- IVD** for in vitro diagnostic use only
- LOT** lot of manufacturing
- REF** code number
-  storage at temperature interval
-  expiration date (year/month)
-  warning, read enclosed documents
-  Read the directions



ISO 9001:2015  
ISO 13485:2016

