INTRODUCTION

1. AutoZyme NEW BUN is a reagent set for determination of urea nitrogen **based on enzymatic UV-Kinetic initial rate method** using urease and glutamate dehydrogenase.
2. AutoZyme NEW BUN is a **single reagent system** using one step procedure.
3. AutoZyme NEW BUN is **linear** upto 250 mg% of urea nitrogen.
4. AutoZyme NEW BUN can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
5. AutoZyme NEW BUN has no influence of bilirubin, haemoglobin and amino acids in serum.

PRINCIPLE

The enzymatic reactions involved in the BUN assay are as follows :

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \alpha\text{-ketoglutarate} + \text{NADH} \xrightarrow{\text{GLDH*}} \text{Glutamate} + NAD^+$$

***GLDH : Glutamate dehydrogenase**

Urea is hydrolyzed to ammonia and carbon dioxide by urease. Ammonia produced reacts with α-ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidised to NAD^+ in this reaction, which is measured as decrease in absorbance at 340 nm. The rate of decrease in absorbance at 340 nm. is directly proportional to BUN concentration in the specimen. The BUN concentration in the specimen is determined by comparing the sample reaction rate to that obtained with a BUN standard.

PREPARATION OF WORKING SOLUTION

Reconstitute reagents as per instruction indicated on individual bottle label to prepare working reagent.

REAGENT STORAGE & STABILITY

The reagent kit should be stored at 2 - 8° C and is stable till the expiry date indicated on the label.

The working solution is stable for **45 days** at 2 - 8°C.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Tris buffer; pH 7.7	50 mmol/l
• α-ketoglutarate	10 mmol/l
• NADH	0.25 mmol/l
• Urease	10 KU/l
• Glutamate dehydrogenase	900 U/l
• Stabilizers and inactive ingredients	

SPECIMEN COLLECTION & PRESERVATION

Serum or plasma should be obtained in the usual manner except that collection tube should be free of ammonium and fluoride ions. Fluoride and ammonium heparin cannot be used as anticoagulants in methods employing urease.

BUN is stable in serum or plasma for 6 days at 2- 8°C and 6 months at -10°C.

PROCEDURE

- ☐ **Reaction type** ..... **UV - Kinetic**
- ☐ **Reaction direction** ..... **Down**
- ☐ **Wavelength** ..... **340 nm.**
- ☐ **Flowcell temperature** ..... **25°C/30°C**
- ☐ **Zero setting with** ..... **Distilled water**
- ☐ **Delay time** ..... **30 seconds**
- ☐ **No. of readings** **2**
- ☐ **Interval** ..... **30 seconds**
- ☐ **Blank adsorbance limit** ..... **≥ 1.000 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Factor** ..... **20 ÷ (Δ Abs. of std.)**
- ☐ **Linearity** ..... **250 mg% BUN**

Manual assay procedure

Prewarm at 25°C/30°C the required amount of working solution before use.

Perform the assay as given below:

1.0 ml procedure

Standard/Specimen	**0.01 ml (10µl)**
Working solution	**1.0 ml**

First carry out the assay of standard. Mix and start stopwatch simultaneously. Record absorbance at exactly 30 seconds after standard addition and then again at 60 seconds.

Subsequently, carry out the assay of the specimen following exactly the same procedure mentioned above.

Calculation

Calculate the change in absorbance (Δ Abs.) of Standard and Specimen(s).

$$\text{Factor} = \frac{\text{Concentration of Standard}}{\Delta\text{ Abs. of Standard}}$$

$$= \frac{20}{\Delta\text{ Abs. of Standard}}$$

BUN mg% = Δ Abs. of Specimen x Factor

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Note : For convenience the specimen/reagent volume ratio can be altered to 20 µl:1.0 ml. In this case the laboratory would have to establish a fresh factor. The linearity of this assay will be 200 mg%.

1. It is recommended that a BUN standard is run with each batch of test.
2. 1 mg of urea nitrogen corresponds to 2.14 mg% of urea.
3. Throughout the assay of standard/specimen a constant temperature should be maintained.

EXPECTED VALUES

BUN : 5 - 21 mg%
UREA : 10 - 45 mg%

The expected range varies from population to population. Normal range mentioned here may be used as a guideline only.

PROCEDURE LIMITATIONS

1. The method is linear upto 250 mg% urea nitrogen. For higher concentrations, dilute the sample by mixing one volume of the sample with one volume of distilled water, repeat the assay and multiply the result by 2.
2. Discard the working solution if absorbance of the same is less than 1.000 O.D. against distilled water at 340 nm.
3. Do not use strongly haemolysed samples.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might effect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

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Quality Assurance - On line testing

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE
European Conformity

AR. No.: I 05 *BN-2009-06-00I*

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AutoZyme

NEW

BUN *Initial Rate*

Note :

Guidelines for Direct Urea Estimation

- Input 42.8 mg% urea as standard value.
- Assay linear upto 535 mg% urea.

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