INTRODUCTION

1. ***Infinite*** Liquid BUN is a reagent set for determination of blood urea nitrogen (BUN) concentration in serum or plasma based on **Enzymatic UV - Kinetic initial rate method.**
2. ***Infinite*** Liquid BUN is a **ready-to-use,** two liquid reagent system.
3. ***Infinite*** Liquid BUN estimates urea nitrogen concentration in just **1minute.**
4. ***Infinite*** Liquid BUN is **linear** upto 250 mg/dl.
5. ***Infinite*** Liquid BUN can be used on any **Spectrophotometer, Discrete Semiautomated and Automated analyzers.** Programme can be designed for any specific analyzer upon request.
6. ***Infinite*** Liquid BUN is **stable till expiry** at 2 - 8°C.

PRINCIPLE

Urea is hydrolysed to ammonia and carbondioxide 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.

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

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

*Abbreviations
GLDH = Glutamate dehydrogenase

PREPARATION OF WORKING SOLUTION

Prepare working solution by mixing **Reagent R_1 and Reagent R_2** in the ratio **4 : 1** as per requirement.

REAGENT STORAGE & STABILITY

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

R_1 and R_2 reagents are stable till expiry at 2 - 8°C.

The working solution (4 R_1 + 1 R_2) is stable for 30 days at 2 - 8°C.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Tris buffer, pH 7.6	120 mmol/l
• Urease	≥ 20 KU/l
• Glutamate dehydrogenase	≥ 1 KU/l
• NADH	0.25 mmol/l
• α-ketoglutarate	10 mmol/l

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can be used. Anticoagulants such as ammonium heparin and fluoride should not be used. Urea nitrogen (BUN) concentration in serum or plasma is stable for 6 days at 2 - 8°C and 6 months at -20°C. The samples should be brought to room temperature, (25 - 30°C) prior to use.

PROCEDURE

☐ Reaction type	UV - Kinetic
☐ Reaction direction	Decreasing
☐ 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 absorbance limit	≥ 1.000 Abs.
☐ Sample volume	0.01 ml (10 µl)
☐ Working solution volume (4 R_1 : 1 R_2)	1.0 ml (1000 µl)
☐ Factor	20 ÷ (Δ Abs. of Standard)
☐ Linearity	250 mg/dl

MANUAL ASSAY PROCEDURE

Prewarm at 25°C / 30°C the required amount of working solution before use. Perform the assay as given below :

1 ml procedure

Standard / Serum / plasma	0.01 ml
Working solution	1.0 ml (800 µl R_1 + 200 µl R_2)

Mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette and start the stop watch simultaneously. Record the first reading at 30th second and subsequently one more reading with 30 seconds interval at 340 nm.

Calculation:

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

Factor = Concentration of Standard ÷ Δ Abs. of Standard
= 20 ÷ Δ Abs. of Standard

Concentration of BUN in mg/dl = Δ Abs. of Specimen x Factor

Note :

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

EXPECTED VALUES

Serum / Plasma

Urea Nitrogen (BUN) : 5 - 21 mg/dl

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range.

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PROCEDURE LIMITATIONS

1. If the BUN concentration exceeds 250 mg/dl, dilute the specimen with normal saline or distilled/delonized water and repeat the assay. The result obtained should then be multiplied with the dilution factor to obtain correct BUN concentration.
2. The working solution is considered unsatisfactory and should not be used if the absorbance is less than 1.000 at 340 nm. against distilled water.
3. Do not use strongly haemolysed samples.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include normal and 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 affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

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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

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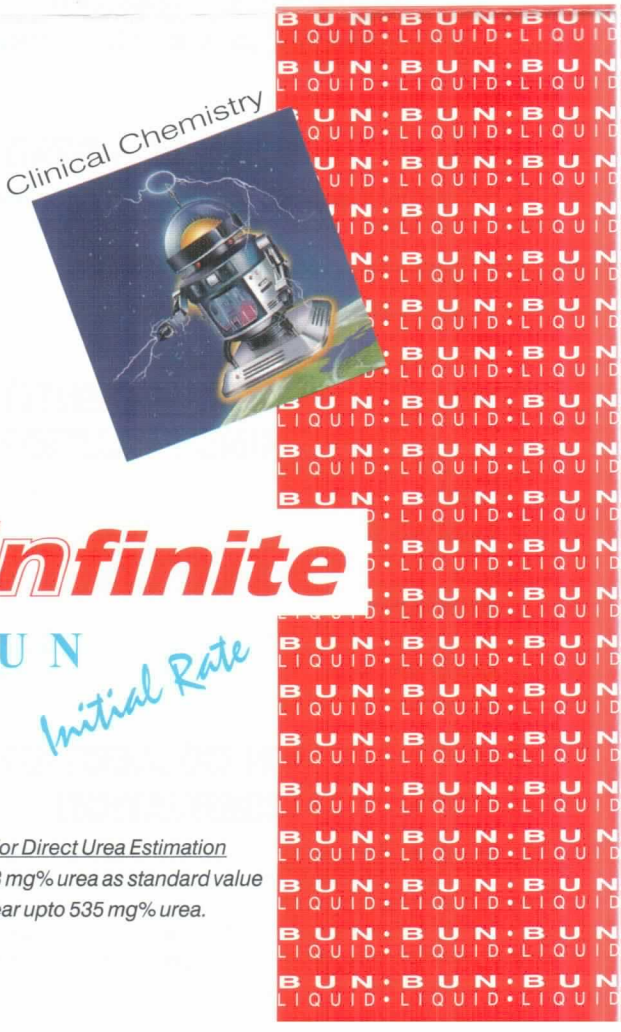
Quality Assurance - On line testing



Infinite

BUN

Initial Rate

NOTE :

Guidelines for Direct Urea Estimation

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