INTRODUCTION

1. AutoZyme Urea is a reagent set for determination of Urea / Blood Urea Nitrogen based on **enzymatic method** using Urease.
2. AutoZyme Urea is a **two reagent system** using two step procedure.
3. AutoZyme Urea is **linear** upto 350 mg%.
4. The **stability** of enzyme solution is 6 months at 2 - 8°C
5. AutoZyme urea can be used on any **Colorimeter, Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
6. AutoZyme Urea has one **step reconstitution.**
7. Urea can be determined in just **8 minutes.**
8. **Accuracy, precision, specificity and sensitivity** is better than that of chemical method like : Diacetylmonoxime (DAM) and NED.
9. The **shelf-life** of AutoZyme Urea is 18 months.

PRINCIPLE

Urease splits urea into ammonia and carbondioxide. Ammonia released in this reaction reacts with hypochlorite and phenolic chromogen to produce green colour. The absorbance of this green colour at 578 nm. (570-620) is directly proportional to the concentration of urea in specimen.

$$NH_2\text{-}CO\text{-}NH_2 \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenolic chromogen} \longrightarrow \text{Green colour}$$

PREPARATION OF ENZYME SOLUTIONS

Reconstitute enzyme & diluent as per instructions indicated on individual bottle label to prepare enzyme solution. Mix gently by inversion. The chromogen solution is ready-to-use.

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 reconstituted enzyme solution is stable for 6 months when stored at 2-8°C. **DO NOT FREEZE THE ENZYME SOLUTION.** The chromogen solution is stable for 6months at 2-8°C after opening.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Phosphate buffer; pH 7.0	35 mmol/l
• Urease	15 KU/l
• Phenolic Chromogen	2 mmol/l
• Hypochlorite	4 mmol/l
• Activators and preservative	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean and dry container (free of NH_3). Avoid the use of plastic or siliconized containers which may prolong clotting time.

Following anticoagulants may be used for plasma separation:

• EDTA	2 mg/ml of blood
• CITRATE	6 mg/ml of blood
• OXALATE	3 mg/ml of blood
• HEPARIN	200 IU/ml of blood

Ammonium salts of anticoagulants and sodium fluoride should not be used as anticoagulant.

Urea in the specimen is stable for a week when stored at 2-8°C and for a month when stored at -10°C.

PROCEDURE

- ☐ **Reaction type** ..... **End-Point**
- ☐ **Reaction time** ..... **3 + 5 min.**
- ☐ **Wavelength** ..... **578 nm. (570 - 620 nm.)**
- ☐ **Zero setting with** ..... **Reagent Blank**
- ☐ **Blank absorbance limit** ..... **< 0.200 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10µl)**
- ☐ **Reagent volume** ..... **1.0 ml + 1.0 ml**
- ☐ **Standard concentration.** ..... **40 mg%**
- ☐ **Linearity** ..... **350 mg/dl**

Manual assay procedure

Prewarm at room temperature (25 - 30°C) the required amount of Enzyme solution and chromogen solution before use.

Perform the assay as given below :

2.0 ml procedure

	Serum/Plasma	Standard	Blank
	0.01 ml	0.01 ml	-
Enzyme Solution	1.0 ml	1.0 ml	1.0 ml
Mix and incubate for three minutes at 37°C.			
Chromogen Solution	1.0 ml	1.0 ml	1.0 ml

Incubation

Mix and incubate the assay mixture at 37°C for 5 minutes. After completion of incubation measure the absorbance of assay mixture against blank at 578 nm. (570-620 nm.). The final colour is stable for 2 hours if not exposed to direct light.

Note :

1. **Reagent to sample ratio can be altered proportionally without affecting the performance of assay.**
2. **Instrument having 3.0 ml reading capacity should carry out assay using following volumes :**

Sample / Standard	0.02 ml	
Enzyme and Chromogen solution	1.5 ml	each

Calculation

$$\text{Urea in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 40$$

$$\text{BUN in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 18.69$$

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EXPECTED VALUES

Urea	:	**10 to 45 mg%**
Urea Nitrogen	:	**5 to 21 mg%**

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

PROCEDURE LIMITATIONS

1. Fluoride as an anticoagulant cannot be used as it inhibits urease activity. Anticoagulants having ammonium ions should not be used because of extreme sensitivity of the colour reaction to ammonia.
2. If the urea value exceeds 350 mg% then dilute the specimen suitably with normal saline. In such case, the results obtained should be multiplied with the dilution factor to obtain the correct urea value.
3. During assay, blank develops a prominent yellow colour. Only if the absorbance of the same exceeds 0.200 at 578 nm. against distilled water, the reagents should be considered unsatisfactory and should not be used.
4. Care should be taken so as not to contaminate the reagents by interchanging the pipettes of enzyme solution & chromogen.
5. Detergents containing ammonium ions & strong oxidizing disinfectants (sodium hypochlorite) should not be used for washing glass wares.

Quality Assurance - On line testing

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

1. Webster, D., ***Clin. Chem. 23,*** 663 (1977).
2. Dumas, B.T. et al, ***Clin. Chem. Acta,*** 31, 87 (1971).
3. ***"Practical Clinical Biochemistry",*** Harold Varley, V edition, Vol. I, pp 457(1980).

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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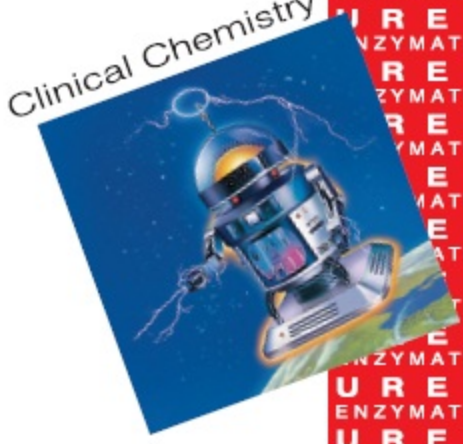
ACCUREX BIOMEDICAL PVT. LTD.
Head Office - Mumbai. Tel.: 91(022) 67446744; Fax: 91(022) 66662667
E-mail: accurex@vsnl.com; Website: www.accurex.org
Plant : G-54, MIDC Tarapur, Boisar, Thane - 401 506. INDIA.



AutoZyme

UREA

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